SHOCK INDUCES A DEFICIT IN THE RECOVERY OF FUNCTION AFTER A CONTUSION INJURY: IDENTIFYING THE RELATIVE CONTRIBUTIONS OF THE BRAIN AND SPINAL CORD

A Thesis

by

ANNE CAROLINE BOPP

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2005

Major Subject: Psychology

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Approved by:

Chair of Committee,	James Grau
Committee Members,	Mary Meagher
	Rajesh Miranda
Head of Department,	Steve Rholes

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ABSTRACT

Shock Induces a Deficit in the Recovery of Function after a Contusion Injury: Identifying the Relative Contributions of the Brain and Spinal Cord.

(August 2005)

Anne Caroline Bopp, B. S., Indiana State University

Chair of Advisory Committee: Dr. James W. Grau

Prior studies have shown that exposure to uncontrollable stimulation can have a variety of adverse consequences on plasticity. For example, as little as 30 min of uncontrollable shock to the tail disrupts both the capacity for instrumental learning and the recovery of locomotor function following spinal cord injury (SCI). Whereas evidence suggests that the disruption of instrumental learning depends on maladaptive plasticity within spinal cord neurons, it is still unknown whether the disruptive effects of shock on locomotor recovery following SCI reflects a brain or spinally-mediated effect. The present experiments address this research question by determining whether shock exposure induces an alteration within the spinal cord of contused rats and testing the effects of disrupting communication between the spinal cord and brain during shock exposure to see if this manipulation protects animals from the effects of shock on locomotor recovery. Experiment 1 found that contused rats transected prior to shock exposure failed to acquire the instrumental response when tested 24 hours later. In addition, contused animals transected after shock exposure also failed to learn when tested, though this effect was less robust. Given the results of Experiment 1, it is plausible that impaired spinal function is sufficient to explain the effects of shock on locomotor recovery. Experiments 2 and 3 addressed this possibility by manipulating

communication between the brain and spinal cord prior to shock exposure. In Experiment 2 intrathecal lidocaine was applied rostral to the injury to temporarily disrupt transmission. In Experiment 3, normal brain function was inhibited with intraperitoneal injection of pentobarbital. Interestingly, both manipulations showed that disrupting normal communication between the spinal cord and brain during shock exposure protected animals from the adverse consequences of shock on locomotor recovery. The data suggest that, following SCI, blocking communication between the brain and spinal cord protects animals from the adverse consequences of uncontrollable stimulation.

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INTRODUCTION

Prior studies have shown that exposure to uncontrollable stimulation can have a variety of adverse consequences on plasticity. Exposure to uncontrollable shock has been shown to undermine learning and performance in a variety of instrumental tasks, a phenomenon known as learned helplessness (Crown & Grau, 2001; Grau et al., 1998; Maier & Seligman, 1976; Overmier & Seligman, 1967; Seligman & Maier, 1967; Weiss & Simson, 1986). Learned helplessness has traditionally been studied in intact animals using an instrumental paradigm such as shuttle-box learning. Prior exposure to uncontrollable stimulation disrupts behavioral plasticity, such that even when the response-reinforcer contingency is reinstated, animals fail to learn to escape an aversive stimulus. Likewise, uncontrollable stimulation adversely affects a variety of physiological processes, including immune function and tumor rejection (Maier et al., 1986; Overmier, 2002; Overmier & Murison, 2000; Sandi et al., 1992; Watkins & Maier, 2005). Interestingly, the adverse consequences associated with uncontrollable shock exposure are not observed after an equivalent exposure to controllable shock, a finding that suggests that the consequences of aversive stimulation are modulated by the variable of instrumental control (Maier & Seligman, 1976; Maier, 1984). The effects of exposure to uncontrollable stimulation on subsequent instrumental learning have been linked to a number of brain systems including the dorsal raphe nucleus and the locus coeruleus, the main centers for the serotonergic and

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noradrendergic systems, respectively. (Grahn et al., 1999; Maier et al., 1995a; Maier et al., 1995b; Weiss & Simson, 1986).

Recently, it has been shown that spinal systems can support instrumental learning (Crown & Grau, 2001; Grau et al., 1996, 1998; Joynes & Grau, 2004). This phenomena has been studied using procedure modeled after a version of the master-yoke paradigm used to study learning in an invertebrate preparation (Horridge, 1962). Using a version of this paradigm, Grau and colleagues have shown that neurons in the lumbar spinal cord can support instrumental learning (Crown & Grau, 2001; Grau et al., 1996, 1998; Joynes & Grau, 2004). Briefly, prior to testing, rats receive a spinal cord transection at the second thoracic vertebra. Approximately 24 hrs later, spinalized rats are suspended above a salt solution and shock electrodes are attached to the rat's hindleg. An insulated contact electrode is then taped to the plantar surface of the rat's foot. A computer program is used to monitor foot position, such that when the electrode attached to the rat's foot contacts the underlying salt solution, a shock is delivered to the tibialis anterior muscle of the hindleg. Shock to the tibialis anterior causes flexion at the ankle joint, lifting the animal's leg out of the water. When using a triadic design, two rats are set up to receive shock simultaneously, though one rat (master) receives shock only when the leg extends, allowing the contact electrode to touch the underlying salt solution (response contingent shock). The other rat (yoke) receives shock every time the master does, independent of its own leg position (noncontingent shock). Within this master-yoke paradigm, both rats receive exactly the same amount of shock, but only one has access to the leg position-shock contingency.

Using this paradigm, we have shown that spinalized animals can acquire an instrumental response, learning to maintain their leg in a flexed position to minimize net shock exposure (Grau et al., 1996; 1998). This is in contrast to animals that receive shock

independent of leg position. Animals given uncontrollable shock do not learn to maintain their leg in a flexed position, and thus fail to acquire the instrumental response. Within this paradigm, perhaps one of the most interesting outcomes was observed when subjects were later tested under common conditions with controllable shock. Master rats demonstrate positive transfer when tested under common conditions, re-acquiring the instrumental task faster, while previously yoked rats failed to learn, a negative transfer effect that resembles the phenomena of learned helplessness (Crown et al., 2002a; Grau et al., 1998). Seeking confirmation that the deficit in instrumental learning is centrally mediated, Crown and colleagues (2002b) found that severing the sciatic nerve prior to uncontrollable shock exposure protected animals from the deficit. Further evidence was provided in support of central mediation when it was shown that intrathecal administration of the sodium channel blocker lidocaine prior to uncontrollable shock exposure prevented animals from developing the instrumental learning deficit (Joynes et al., 2003).

Following the initial demonstration by Grau and colleagues (1998) subsequent studies were conducted to delineate the nature of the noncontingent shock-induced behavioral deficit. The studies in our spinal model revealed some interesting parallels to the learned helplessness deficits observed in intact animals. We showed that 30 min of intermittent 1.5 mA uncontrollable shock to either the leg or tail disrupts instrumental learning, an effect that decays after 48 hrs (Crown et al., 2002a). This parallels the time course observed in intact subjects (Maier & Minor, 1993). In addition, the learning deficit seen in intact and spinalized rats can be ameliorated by prior, or subsequent, exposure to controllable stimulation (so named immunization and therapy effects) (Crown and Grau 2001; Seligman et al., 1968; 1975). We have also determined that the shock-induced deficit in spinalized rats involves the release of opioid peptides. Joynes and Grau (2004) showed that both systemic and intrathecal administration of the general opioid antagonist,

naltrexone (7-14 ug/uL) given prior to, or immediately following, noncontingent shock exposure blocked the induction of the deficit (a form of neurochemical immunization). Likewise, naltrexone blocks the induction of the deficit in intact rats (Maier et al., 1980). Further studies in spinalized rats identified that only the kappa opioid antagonist, norBNI, was effective in restoring learning in rats that had previously been exposed to noncontingent shock (Joynes & Grau, 2004).

Recognizing that these findings could have important clinical implications, Grau and colleagues have begun to examine the effects of uncontrollable stimulation on another measure of plasticity, the recovery of function following spinal cord injury. In studying spinal cord injury, we have employed the most clinically relevant model for studying injury, the spinal contusion model (Young, 2002). Using this model, we have shown that as little as 6 min. of uncontrollable stimulation hurts locomotor recovery, while equal exposure to controllable stimulation has no effect (Grau et al., 2004). Locomotor recovery was evaluated primarily using the BBB Scale, a 21-point scaled that is sensitive to changes in spontaneous locomotor recovery of the hindlimbs (Basso et al., 1995). BBB scores were greatly impacted by shock treatment, with shocked animals temporarily exhibiting a significant decline in locomotor ability and a slower recovery that reaches asymptote at a lower level (Grau et al., 2004). Exposure to uncontrollable stimulation, however, only disrupts recovery when it occurs within a week of injury, effectively elucidating a critical window for exposure. Uncontrollable stimulation also affected several other measures of recovery. Shocked animals exhibited decreased reactivity to mechanical and thermal stimuli (indicating a decrease in sensory function), impaired autonomic function (evident in an increased latency to recovery bladder control), increased incidence of hindlimb spasticity, neuropathic pain behaviors (autophagia), and increased mortality. Shock also results in a decrease in remaining spinal cord tissue at the injury site (both grey and white

matter), presumably due to enhanced apoptotic and necrotic cell death, and an increase in lesion size (Grau et al., 2004; Liu et al., 2003).

At present, it is not clear whether the consequences of uncontrollable stimulation on recovery reflect a brain- or spinally-mediated phenomena. In our preparation, shock is presented caudal to a moderate contusion injury that should attenuate communication with the brain. However, some communication with the brain is preserved and it is known that descending fibers, which originate in the brain and brainstem, can inhibit the development of the deficit (Crown & Grau, submitted). The current experiments addressed these issues by seeking evidence that shock exposure induces an alteration within the spinal cord of contused rats and examining whether disrupting brain function during shock exposure alters the effect of shock exposure on recovery. If the effects of uncontrollable stimulation are due solely to an alteration in spinal cord plasticity, these manipulations should not eliminate the deficit. In fact, blocking communication from the brain could potentiate the deficit by disrupting any surviving brain-mediated protective effects. Alternatively, if the effects of uncontrollable stimulation reflect a brain dependent process, these manipulations should attenuate the consequences of shock exposure.

GENERAL METHODS

Subjects

Subjects were male Sprague-Dawley rats (Rattus norvegicus) obtained from Harlan (Houston, TX). All rats were approximately 94-100 days old (350-450 g) at the start of testing and were individually housed, with food and water continuously available. Rats were maintained on a 12-hr light-dark cycle, with all behavioral testing performed during the light portion of the cycle.

Surgery

Contusion Surgery. Subjects received a contusion injury using the MASCIS device developed by Gruner (1992) and Constantini and Young (1994). First, subjects were anesthetized with pentobarbital (50 mg/kg, i.p.). Ten min later, spinal reflexes were assessed to verify that a stable, and comparable, level of anesthesia was achieved. The injury site was then shaved, disinfected with iodine, and a 7.0 cm incision was made on the animal's back. Next, two incisions were made on either side of the spinal column, and the vertebrae dorsal and medial to T10-T11 was cleared and the spinal tissue exposed. The vertebral column was then fixed within the MASCIS device and a moderate injury produced by allowing the 10 g impactor (outfitted with a 3.0 mm tip) to drop 12.5 mm. After injury, the subjects were removed from the device, placed on a heating pad, and the wound closed with Michel clips. To help prevent infection, subjects were treated with 100,000 units/kg Pfizerpen (penicillin G potassium) immediately after surgery and again 2 days later.

Subjects in Experiment 2 had intrathecal cannulae lowered into the upper thoracic region of the spinal cord using the procedure modified from Yaksh and Rudy (1976). A segment of polyurethane tubing (18.5 cm; PE-10, Becton Dickexnson, VWR) fitted with a 0.23-cm (diameter) stainless steel wire (SWGX-090, Small Parts Inc.) was inserted 4 cm

rostral to the laminectomy at vertebral level T11. The tubing was then inserted into the subarachnoid space, between the dura and the white matter, so as to lie on the dorsal surface of the cord. The exposed end of the tubing was secured to the adjacent tissue using Superglue. The wire was then pulled from the tubing and the wound caudal to the exposed length of tubing was closed using Michel clips.

During recovery, hydration was maintained with supplemental injections of saline, and the rat's bladder was expressed at regular intervals. Michel clips were removed 14 days after surgery. At the end of behavioral testing, subjects were euthanized with pentobarbital (100 mg/kg).

Transection Surgery. For Experiment 1, the spinal cord was transected at the eighth thoracic vertebra (T8) as described in Grau et al. (1998). Briefly, rats were anesthetized with pentobarbital (50 mg/kg). The tissue over T8 was cleared away and the cord was transected using microscissors. The exposed cord was then covered with Oxycel (Parke-Davis) and the wound closed with Michel clips.

Apparatus

Shock Treatment. In all experiments uncontrollable tailshock was applied while subjects were restrained in Plexiglas tubes [22 cm (l) x 6.8 cm (int. dia.)]. A sheet of Plexiglas formed a floor, 5.5 cm wide (lying 5.3 cm from the top of the tube) on which the rats lied. Tailshock was generated using a 660-V AC transformer (with a large series resistance) and applied through electrodes constructed from a modified fuse clip. The metal plates of the clip were covered with electrode paste (Harvard Apparatus) and taped 15 cm from the base of the rat's tail (for additional details see Crown et al., 2002a). Shock was applied by attaching one lead from a BRS/LVE shock generator (Model SG-903) to each electrode.

Instrumental Testing. Instrumental training was conducted while spinal rats were loosely restrained in tubes [23.5 cm (l) x 8 cm (int. dia.); see Grau et al., 1998, Fig. 1]. Two slots [5.6 cm (l) x 1.8 cm (w)] were cut 4-cm apart and 1.5 cm from the end of the tube, allowing both hind legs to hang freely. Legshock was applied by attaching one lead from a BRS/LVE shock generator (Model SG-903) to a wire inserted through the skin over the tibia, 1.5 cm from the tarsals. The other lead was attached to a 2.5-cm stainless steel pin that was inserted 0.4 cm into the tibialis anterior muscle 1.7 cm above the other electrode. Leg position was monitored using a contact electrode constructed from a 7-cm 0.018" stainless steel rod that was taped to the foot. The last 2.5 cm of the electrode was insulated from the foot with heat shrink tubing. The rod was taped to the plantar surface of the rat's foot with the end positioned directly in front of the plantar protuberance. A fine wire extended from the rear of the rod and connected to a digital input that was monitored by a Macintosh computer. A plastic rectangular dish containing a NaCl solution was placed approximately 7.5 cm below the restraining tube and a ground wire was placed in the solution. When the contact electrode attached to the rat's paw touched the solution, it completed the circuit monitored by the computer. Flexion force was measured by attaching a monofilament plastic line to the rat's foot immediately behind the plantar protuberance. The line was passed through an eyelet positioned under the paw and attached to a strain gauge. After the line was connected to the rat's paw, the ringstand was positioned so that the line was taut, just barely triggering the gauge. For additional details see Grau et al. (1998).

Locomotor Recovery. In Experiments 2 and 3, locomotor behavior was assessed in an open enclosure [a 99.1 (diameter) x 20.3 (deep) cm blue children’s wading pool].

Behavioral Testing. In Experiments 2 and 3, animals were tested at the end of the recovery period using the Beamwalk apparatus as described by Hicks and D'Amato (1975) and the Ladder Beam apparatus as described by Soblosky and colleagues
(Soblosky et al., 1997)

Mechanical Reactivity. The same Plexiglas tubes used for instrumental testing were used to restrain the rats during tactile reactivity assessment. Tactile reactivity was assessed using von-Frey filaments (Stoelting Co., Chicago, IL) and applied to the plantar surface of the paw.

Pain Reactivity. Reactivity to both thermal and shock stimuli was tested 24h after tactile testing using the apparatus and procedures described in King et al. (1996). Briefly, thermal reactivity was tested using a 375-W movie light focused on the rat's tail by means of a condenser lens positioned 8 cm below the light source. Shock thresholds were assessed using a manual shocker (BRS/LVE, Model SG-903) that allowed continuous variation of shock intensity between 0 and 2-mA (AC, constant current). Test shocks were applied 7 cm from the base of the tail by means of electrodes constructed from lightweight fuse clips. Test shock intensity was gradually incremented at a rate of 0.05 mA every 3 s. For testing shock and thermal reactivity, the subject's tail was positioned in a 0.5 cm deep groove cut into an aluminum block. Plastic sides (6.0 cm x 6.7 cm) were placed along the sides of the aluminum block to maintain the rat's tail under the heat source. An insulated 10 cm wire hook was taped to the last 2.5 cm of the rat's tail. The hook was placed over an elastic band located 11 cm behind the aluminum block. The flexibility of the elastic band allowed for a tail-flick response while maintaining the rat's tail under the heat source. A photocell, located in the groove of the aluminum block, was used to automatically detect whether the rat moved its tail laterally 0.5 cm. To activate the photocell in the absence of

radiant heat (on the shock test trials), a small 28-V light (General Instrumental, 1820) was positioned 3.5 cm above the photocell.

The latency to vocalize was assessed using a microphone located at the front end of the tube. The vocalization threshold was set to 80 dB. A computer (Apple, Macintosh 8500) monitored the circuit controlled by the photocell and the output intensity from the microphone. After both movement and vocalization responses were detected, the shock or heat was terminated. If a subject failed to respond, the test trial was automatically terminated after 8-s of heat exposure or after shock intensity reached 1.2 mA.

Behavioral Procedures

Shock Treatment. Shock treatment was administered 24 hrs after the contusion injury (Day 1), and after locomotor behavior was scored. Subjects were placed in the restraining tubes and the tail electrodes were secured with porous adhesive tape. Subjects in all experiments received either 1800 s of uncontrollable tailshock or an equivalent period of restraint. The shocks were 1.5 mA, 80 ms in duration, and occurred on a variable time schedule (range 0.2 to 3.8 s) with a mean interstimulus interval of 2 s.

Instrumental Testing. In Experiment 1, instrumental testing was initiated approximately 24 hrs after shock treatment. Before the rats were placed in the restraining tubes, their rear legs were shaved and marked for placement of the shock leads. The wire electrode was then inserted over the tibia at the distal mark and the rats were placed in the restraining tubes. Next, the contact electrode used to monitor leg position was taped to the paw. To minimize lateral movements of the tibia and fibula, a 20-cm piece of porous tape (Orthaletic, 1.3 cm) was wrapped around the ankle and taped to a bar extending across the apparatus directly under the front panel of the restraining tube. One lead from the shock generator was attached to the stainless steel wire inserted over the tibia. The shock generator was set to deliver a 0.1 mA shock and the region over the second mark was

probed to find a site that elicited a vigorous flexion response. The pin was then inserted perpendicular to the body into the tibialis anterior muscle. After the line connected to the strain gauge was placed over the rat's paw, we verified that a single intense (1.6 mA) test shock (0.3 s) elicited a flexion response of at least 0.8 N. Shock intensity was then adjusted so that a 0.3-s shock produced a flexion force of 0.4 N. The plastic line to the strain gauge was then removed. Finally, three 0.15-s legshocks were administered, spaced about 1 s apart, to establish the tarsus' resting position, and the height of the solution was adjusted so that the tip of the rod was submerged 4 mm below the surface. During testing the shocks were 80 ms in duration and occurred on a variable time schedule with a mean of 2 s (range: 0.2 s to 3.8 s). All subjects underwent instrumental testing for 1800 s (30 min).

Response duration was used as our primary index of learning. Response duration is calculated as (time out of solution)/(no. of responses + 1) for each 60 sec time bin. Normally, transected animals tested under response contingent shock in the instrumental learning apparatus show a progressive increase in response duration. Response number (defined as the number of responses an animal makes in a given 60 sec time bin) is another index of learning we commonly measure in the instrumental task. As an animal learns the relationship between leg position and shock, they begin to maintain their leg out of water for a progressively longer period of time, and as a result, make less leg flexion responses to the shock.

Locomotor Recovery. Recovery of hindlimb stepping was assessed while subjects were able to move freely about an open field. Because rodents often remain motionless (freeze) when first introduced to a new apparatus, subjects were acclimated to the observation fields for 5 min per day for 4 days prior to surgery. The first behavioral assessment was conducted 24 hrs after surgery, and prior to shock treatment. Each subject was placed in the open field and observed for 4 min. During this period, locomotor

behavior was scored using the procedure developed by Basso, Beattie, and Bresnahan (BBB Scale, 1995). Care was taken to ensure that the investigators scoring behavior had high intra- and inter-observer reliability (all r's > 0.94) and that they were blind to the subject's experimental treatment. In Experiments 2 and 3, locomotor behavior was scored once per day for 1 week (Days 1-7), every other day from Day 7 to Day 15, and then every 3rd day from Day 15 to Day 21. A video record of each subject's performance in the open field was obtained on Days 1, 2, 4, 7, 14, and 21.

A transformation was applied to locomotor scores to insure that the data would be more adaptable to parametric analyses (Ferguson et al., 2004). This transformation pools BBB scores to eliminate a discontinuity in the scale (scores 2-4 become a single transformed score of 2) as well as combining scores in the late phase of recovery that are infrequently used in a moderate contusion injury model (scores 14-21 become a single score of 12). As a result of applying this transformation, the scores become more continuous, the interval duration between scores becomes more equivalent, and as a result, the scale more closely approximates ordinality. With these criteria in place, we can apply metric operations (computation of mean performance across legs), with an improved justification for parametric statistical analysis that generates increased statistical power.

Behavioral Testing. All subjects in Experiments 2 and 3 were tested at the end of the recovery period using the Beamwalk test adapted from Hicks and D'Amato (1975) and the Ladder Beam test adapted from Soblosky and colleagues (Soblosky et al., 1997).

Mechanical Reactivity. Sensory function was assessed after Day 21. Progressively stronger tactile stimuli were applied sequentially at approximately 2 s intervals until subjects exhibited a paw withdrawal (motor response) and vocalized. If one or both responses were not observed, testing was terminated at a force of 2.9 N. Each subject was tested twice on each foot in a counterbalanced ABBA order. Test sequences were spaced 2

min apart. Stimulus intensity was recorded using the formula provided by Semmes-Weinstein: Intensity = log10 (10,000 * g force).

Pain Reactivity. On an alternate day (test order was counter-balanced across groups) nociceptive reactivity was assessed using stimuli (a gradually incremented shock and radiant heat) and procedures employed in prior studies (e.g., Crown et al., 2000; King et al., 1996; McLemore et al., 1999). Briefly, subjects were placed in the restraining tubes and the apparatus used to assess nociceptive reactivity was attached to the tail. Next, subjects were acclimated in the apparatus for 15 min. Thermal and shock thresholds were then assessed at 2 min intervals, 2 times each, in an ABBA order.

Histology

Following behavioral testing, subjects in all experiments were overdosed with pentobarbital (100 mg/kg) and perfused intracardially with 60 ml of 0.9% saline and 160 ml of 4% paraformaldehyde. Experiments 2 and 3 subjects' spinal cords (segmentsT13-S4) were then removed and postfixed in paraformaldehyde before being transferred to paraffin for sectioning. A microtome was used to cut 10 um sections and the tissue was placed on slides for subsequent immunohistological staining. Luxol Fast Blue and Cresyl Violet staining were used to assess the extent of damage and identify lesion characteristics. Quantification of the results of these histological examinations was performed using Canvas 8.0 software for MacIntosh. Total cross-sectional area of the cord and spared tissue was assessed at the lesion center from camera lucida drawings made by an experimenter who was blind to the subject's treatment condition. Sections 600 µm from the lesion center (rostral and caudal) were also drawn and analyzed. Four indices of lesion magnitude were derived from the camera lucida drawings: lesion area, area of residual gray matter, area of residual white matter, and width. For derivation of lesion area, and the spared gray/white matter, the camera lucida drawings were scanned onto a Macintosh

computer and imported into CANVAS 8 (Deneba systems Inc.). To determine lesion area (number of pixels), an observer who was blind to the experimental treatments traced around the boundaries of cystic formations and areas of dense gliosis (Basso et al., 1996). Nissl-stained areas that contained neurons and glia of approximately normal densities denoted residual gray matter. White matter was judged spared in myelin-stained areas lacking dense gliosis and swollen fibers. The total area of each cross-section was derived by summing the areas of the lesion, gray and white matter. Width was determined from the most lateral points along the transverse plane. These analyses yielded six parameters for each section: white matter, gray matter, spared total tissue (white + gray), lesioned tissue referred to as damage, net area (white + gray + damage). Additionally, corrected parameters were then applied to formulas that allowed us to assess the amount and nature of the spared tissue, as well as to quantify the total extent of the lesion by assessing both visible damage and missing tissue. As the corrected values require the use of a standardized width coefficient, raw width scores were first compared to assure no differences between groups [correction factor = width of an undamaged section (standardized)/section width]^2. Provided that there were not any group differences in width, the additional measures were standardized to decrease variability, and as a result, increase statistical power. These corrected values are then applied to formulas to yield a new set of histological outcome measures that were amenable to statistical analysis and include Relative (Rel.) % White, Rel. % Gray, Rel. % Lesion, Rel. % Damage, and Rel. % Missing. Rel. % White and Gray were calculated as (corrected white/std. white) X 100 and (corrected gray/std. gray) X 100. Rel. % Damage was calculated as [corrected damage/(sum of std. white + std. gray)] X 100, which yields the amount of visibly lesioned tissue. Rel. % Lesion was calculated as [(sum of std. white + std. gray) - (section white + gray)]/(sum of std. white + std. gray) X 100. This value identifies the total extent of lesion

by accounting for both missing tissue and damaged tissue. Rel. % Missing was calculated as (Rel. % Lesion – Rel. % Damage), which allowed us to identify the amount of tissue missing from the section.

Statistics

All data was analyzed using repeated measures analysis of variance (ANOVA). Post hoc analyses were conducted using the Duncan's New Multiple Range test. All non-parametric analyses (e.g. incidence of spasticity, mortality, and autophagia) were conducted using a Fisher's Exact Probability test. An alpha value of .05 or below was considered significant.

EXPERIMENT 1

We have previously shown that uncontrollable stimulation inhibits the capacity for instrumental learning, a measure of spinal cord plasticity. We have also shown that brain mechanisms inhibit the development of this deficit, and that this effect depends on fibers that descend through the dorsalateral funiculus (Crown & Grau, 2002). It remains unclear, however, whether brain mechanisms play a role in modulating the deficit in the contusion model, as the injury is known to partially damage descending pathways. The present experiment examines this issue by testing whether contused shocked rats exhibit a spinally-mediated learning deficit. This question was addressed by cutting the spinal cord 2 hrs after contused rats received uncontrollable shock. A second group that received an identical transection prior to shock exposure served as a control and was also tested for instrumental learning. If shock induces a spinally mediated deficit in contused rats, both shocked groups should do poorly relative to the unshocked controls. However, if descending fibers retain some function after injury, rats transected after shock exposure should exhibit a reduced deficit.

Procedure

Forty rats were used in Experiment 1. Subjects were randomly assigned to conditions. Experiment 1 was conducted using a 2 (transection 15 min after contusion X transection 2 hr after shock) X 2 (shock X unshock) factorial design. All subjects received a contusion injury as described in the methods section. Half of the subjects were transected 15 minutes after the contusion injury. Twenty-four hours later, subjects were placed in the shocking apparatus and administered either 30 min of uncontrollable tailshock or an equivalent period of restraint. The remaining half of the subjects were transected 2 hours after shock treatment. Approximately 24 hrs later, all subjects were

tested in the instrumental apparatus (as described in Methods section) with response contingent shock.

Results

To ensure that variation in both the shock intensity needed to induce a 0.4 N change in flexion force and the duration of the first response did not influence either of our behavioral outcomes, an ANOVA was performed on these measures. Results indicated that there were no significant differences in the shock intensity needed to induce a 0.4 N change in flexion response or 1st response duration across groups [Both F's < 1.0, $p > 0.05$].

We found the shock exposure had an effect on spinal cord function independent of whether subjects were transected before or after a contusion injury. Importantly, the magnitude of the deficit was less in animals contused after shock exposure. These results suggest that shock exposure does affect spinal function in contused rats, and that this effect likely develops because the injury disrupts descending fibers. That the deficit is less than that observed in rats shocked after a complete transaction suggests that surviving fibers do retain some protective capacity. Fig. 1 shows that regardless of time of transection, unshocked animals acquired the instrumental response, while animals exposed to uncontrollable shock did not acquire the instrumental response. An ANOVA conducted on the response duration data confirmed that there was a main effect of shock condition [F (1,35) = 31.04, $p < 0.05$]; unshocked animals showed the expected increase in response duration, whereas shocked animals failed to show this increase. Time of transection (immediate or 2 hrs) did not have a significant impact on response duration [F (1,35) < 1.0, $p > 0.05$]. A significant shock X transection time interaction indicated that time of transection moderated the effects of shock on response duration [F (1,35) = 6.11, $p < 0.05$]. Subsequent post hoc analyses indicated that both shocked groups were different from both unshocked groups, $p < 0.05$. No other differences reached significance, $p > 0.05$.

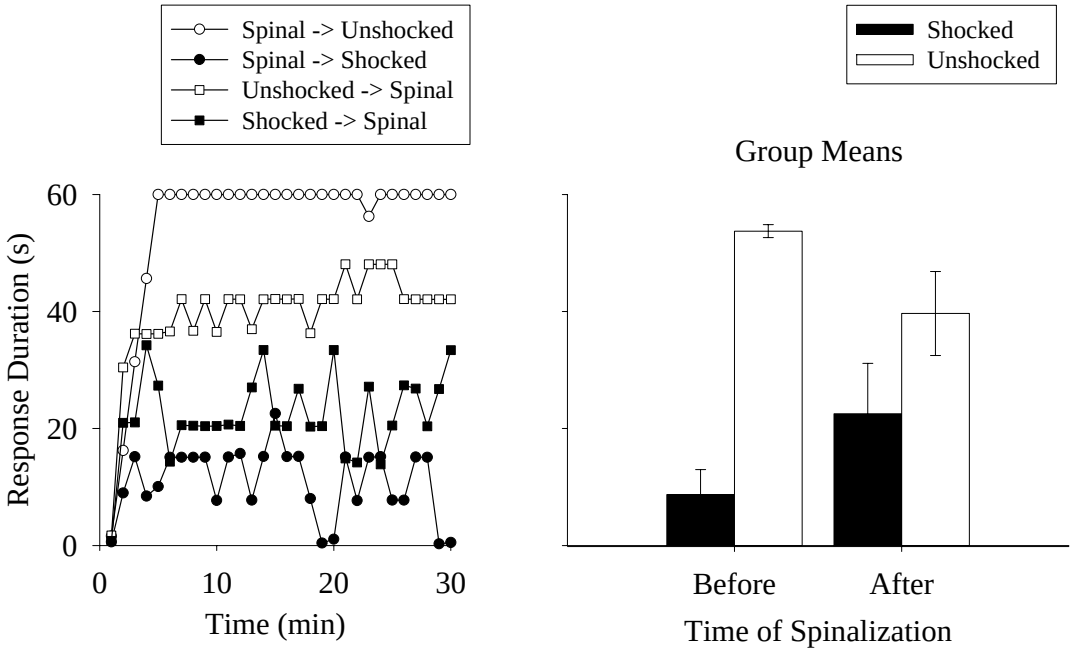

FIG. 1. Impact of shock and transection time on learning

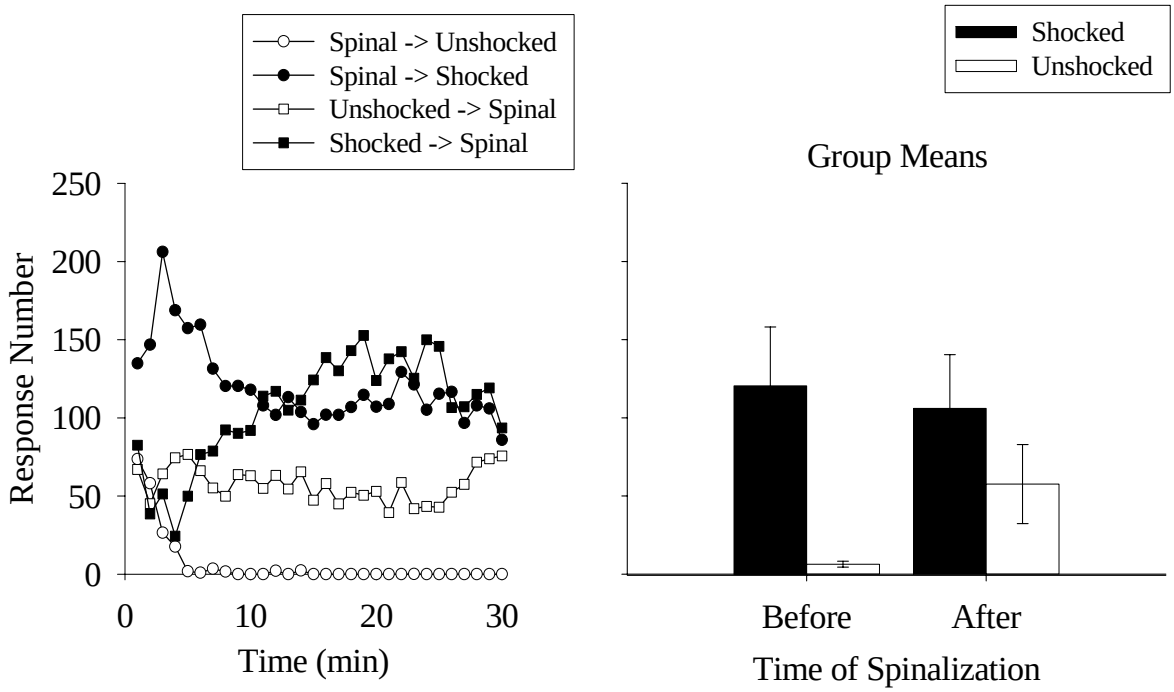

FIG. 2. The impact of shock and transection time on response number

As seen in Fig. 2, both groups of animals exposed to shock, regardless of time of transection, made more responses, and did not decrease their number of responses over the 30 min test session, indicating that they did not learn [F (1,35) = 8.25, $p < 0.01$]. Both groups of unshocked animals show a decreased response number over the test session, with the immediate transection shocked group performing the best and making few responses after min 5 of the test session. There was no significant main effect of transection time [F (1,35) = 0.423, $p > 0.05$], and no significant shock X transection time interaction [F (1,35) = 1.35, $p > 0.05$].

EXPERIMENT 2

Experiment 1 showed that uncontrollable stimulation impacts spinal function in contused rats. Experiments 2 and 3 examined whether this effect is sufficient to explain the effect of shock exposure on locomotor recovery. It remains possible that the deficit in recovery reflects a brain mediated effect, potentially related to examples of learned helplessness. In both experiments, we address this issue by disrupting the brain's capacity to process afferent signals during the period of uncontrollable stimulation. Experiment 2 accomplishes this by blocking neural conduction rostral to the injury through the sodium channel blocker lidocaine. If surviving descending fibers play a protective role, disrupting neural transmission should potentiate the shock-induced disruption of recovery. Alternatively, if brain mechanisms play an essential role, the spinal block should attenuate the locomotor deficit observed after uncontrollable stimulation.

Procedure

Forty rats were used in Experiment 2. The day following a contusion injury, subjects' locomotor performance was scored. Day 1 scores were then counterbalanced to allow each subject to be assigned to one of four conditions. Experiment 2 was conducted using a 2 (lidocaine X PBS) X 2 (shock X unshock) factorial design, consisting of four conditions: Lidocaine Shocked, Lidocaine Unshocked, Phosphate buffered saline (PBS) Shocked, PBS Unshocked. Approximately 24 hrs after contusion injury subjects were placed in the shocking apparatus as described above and the cannula threaded through a small opening in the tube to allow for administration of lidocaine solution (10%) or vehicle. Subjects received 30 µl lidocaine solution (10%) or vehicle through an infusion pump at a rate of 5 µl per min to provide a temporary transection at the T4 segment of the spinal cord. Subjects were then assessed for vocalization response and a spinal reflex to

tail pinch to assure signaling between the brain and spinal cord was disrupted. If lidocaine provided a successful block, animals would retain their spinal reflexes, but would not vocalize to stimulation. Subjects that vocalized or lacked a spinal reflex to the tail pinch were discarded *a priori*. Following assessment, all subjects received 15 µl of drug or vehicle intrathecally at a rate of 0.5 µl per minute during 30 minutes of uncontrollable tailshock or an equivalent period of restraint. Following shock treatment, rat's locomotor recovery was monitored using the BBB scale for 21 days. At the end of recovery all rats were assayed using the additional outcome measures as described in the methods.

Results

Experiment 2 found that a spinal block restored recovery of function suggesting that brain mechanisms play a critical role in mediating the adverse effects of uncontrollable stimulation. Of potential concern is that intrathecal lidocaine applied to the lumbosacral spinal cord blocks the induction of the leaning deficit seen in spinalized animals (e.g, Joynes et al, 2003) possible indicating that the drug's effect in the present experiment was partially a result of blocking intraspinal conduction. Observations argue against this interpretation. First, we verified that the spread of the lidocaine was limited to 1 cm of the cord (specifically extending approx. 1 cm in either direction of T4). Second, the injury would likely reduce spread to more caudal regions.

Impact of Lidocaine Pretreatment on Locomotor Recovery

The impact of lidocaine treatment prior to shock exposure on locomotor recovery is illustrated in Fig. 3. The transformed BBB score values are given on the left y-axis. One day after the moderate injury (Day 1), subjects exhibited a behavioral score of approximately 3. A score of 3 is given when subjects can only exhibit extensive movement of 2 joints, while there is no movement in the third joint. This is within the range of behavior exhibited by subjects that have received a complete spinal transection (Basso et

al., 1996). After Day 3, saline unshocked rats recovered some locomotor function and over the next two weeks reached a behavioral score of 9. Subjects in this range of locomotor scores exhibit occasional weight-supported plantar steps with occasional forelimb-hindlimb coordination. Saline treated shocked rats exhibited deterioration in performance on Days 2-4, which was followed by a slow and stunted recovery. Again, performance reached asymptote at about 2 weeks, but for shocked rats terminal performance was far worse with a locomotor score of approximately 4 (transformed; 6 untransformed). Subjects at this level of locomotor performance can exhibit extensive movement of 2 hindlimb joints and slight movement of the third, but show no signs of sweeping (a precursor to stepping) or weight-supported stepping.

Pretreatment with lidocaine prevented the adverse consequences of shock on recovery after spinal cord contusion injury. An ANOVA confirmed that the behavioral scores on Day 1, prior to shock treatment, did not differ [F (1,35) < 1.0, $p > 0.05$]. A mixed ANCOVA (with Day 1 locomotor performance serving as the covariate) showed that shock treatment had a significant impact on overall performance [F (1,35) = 6.11, $p < 0.05$]. In addition, a significant shock X drug interaction indicated that lidocaine treatment attenuated the effects of shock on recovery [F (1,35) = 9.30, $p < 0.01$]. Post hoc comparisons of the group means indicated that both groups that received lidocaine treatment, as well as the saline unshocked control animals had significantly higher BBB scores when compared to saline shocked animals, $p < 0.05$. No other differences were significant, $p > 0.05$.

To verify that the groups differed at the end of behavioral testing, a separate ANCOVA was performed on the mean performance collapsed across the last 4 days of scoring (Days 13, 15, 18, 21). There were no significant main effects of shock or drug treatment [Both F's < 2.27, $p > 0.05$], but there was a significant shock X drug interaction

[F (1,35) = 10.13, $p < 0.01$]. Post hoc comparisons indicated that the lidocaine shocked and the saline unshocked groups had significantly higher BBB scores when compared to the saline shocked group, $p < 0.05$. No other differences approached significance, $p > 0.05$.

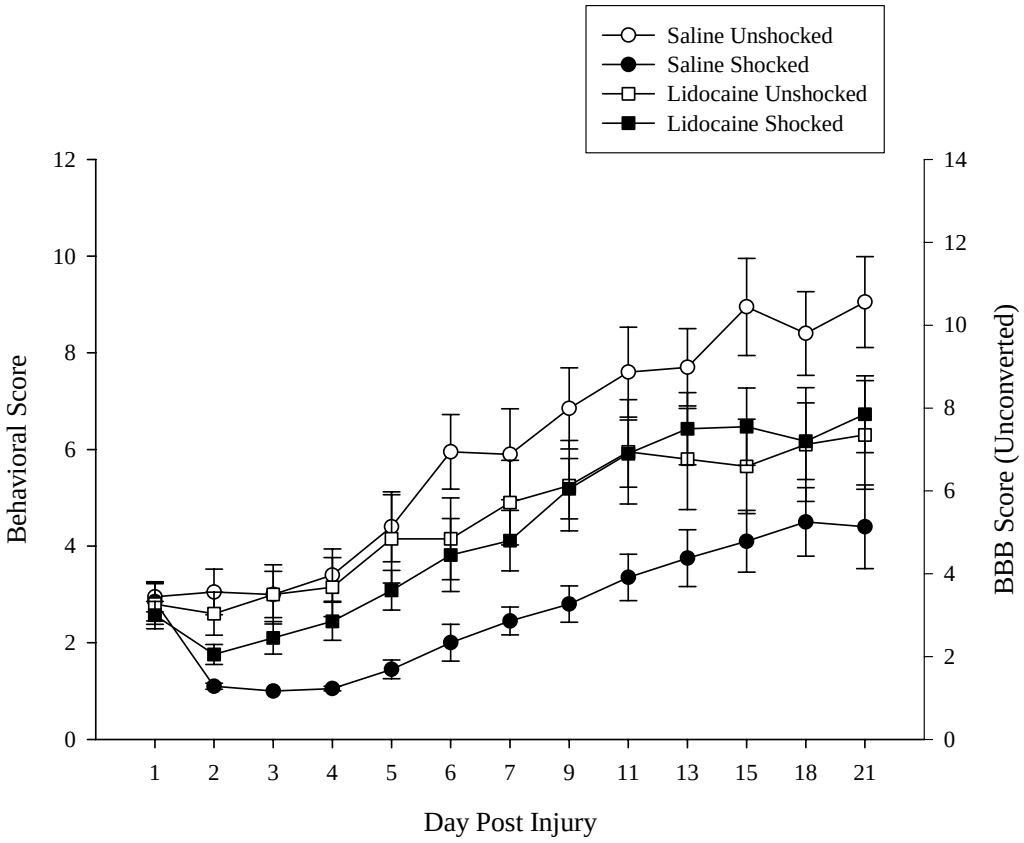

FIG. 3. The effect of lidocaine pretreatment prior to shock on recovery of function

As seen in Fig. 4, lidocaine pretreatment did not significantly protect animals from the harmful effects of shock on beamwalk performance. At the end of the recovery period, animals were tested on the ladder beam and the beamwalk tests. An ANOVA conducted on

the beamwalk data indicate that there was not a significant effect of lidocaine pretreatment or shock [both *F*'s < 1.0, $p > 0.05$], but the interaction between lidocaine pretreatment and shock approached significance [F (1,35) = 3.38, $p = 0.07$].

Fig. 5 shows that shock had a detrimental effect on ladderbeam performance. An ANOVA conducted on the ladderbeam data indicated that there were no main effects of lidocaine pretreatment or shock [both *Fs* < 2.0, $p > 0.05$], but there was a significant interaction [F (1,35) = 4.71, $p < 0.05$]. Post hoc analyses indicated that saline shocked animals made significantly more errors, resulting in a lower score, when compared to the all other groups, $p < 0.05$. No other differences were approached significance, $p > 0.05$.

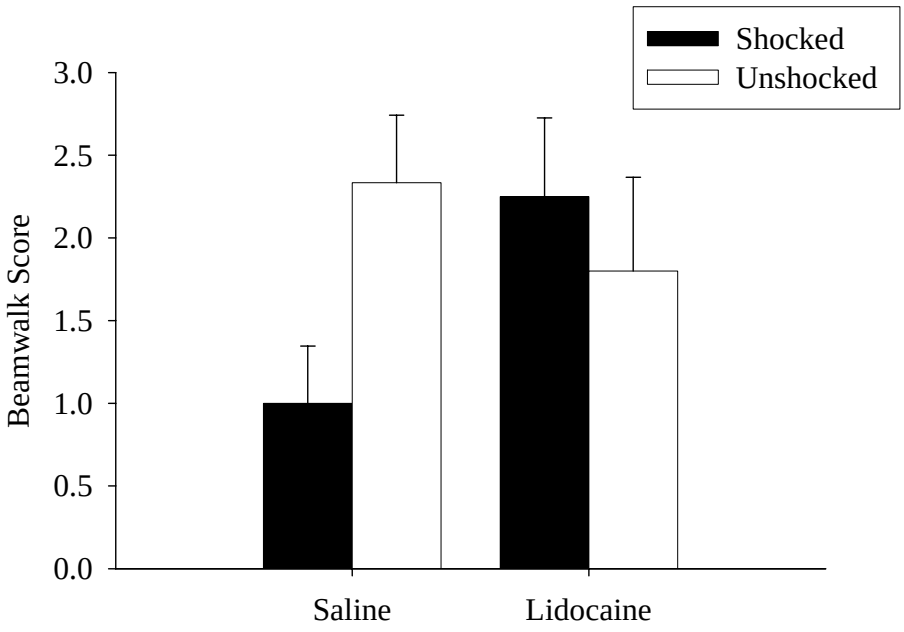

FIG. 4. Effect of lidocaine pretreatment and shock on beamwalk test performance

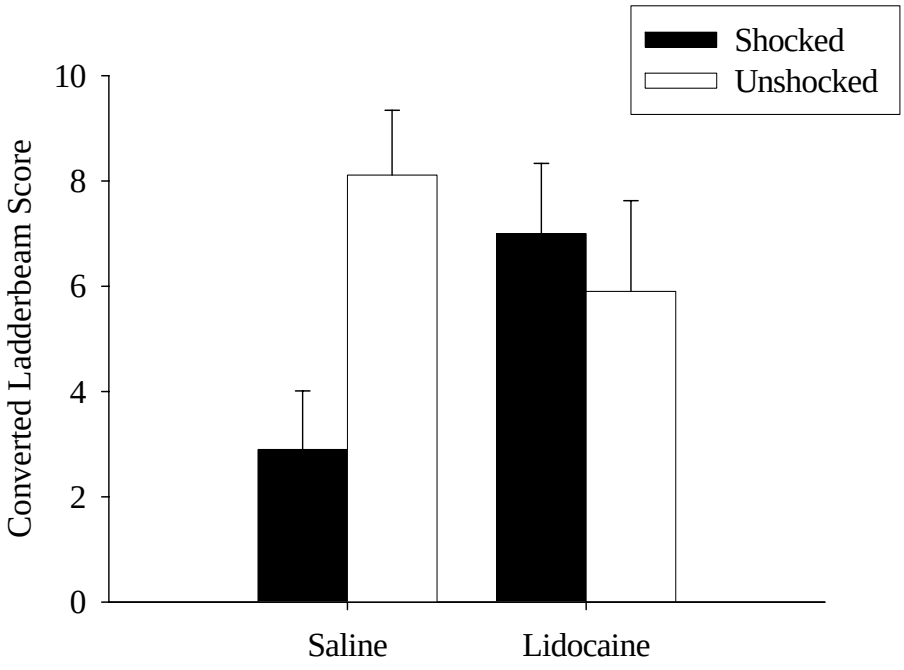

FIG. 5. Effect of lidocaine pretreatment and shock on ladderbeam performance

Other Indices of Recovery

Mortality. Subjects were assigned to the various experimental procedures 1 day after surgery. At this point, the probability of long-term survival was relatively high. Indeed, none of the rats assigned to the lidocaine shocked group or the saline groups died over the 3-week recovery period. There was, however, some mortality in the lidocaine unshocked group. Of the 14 total animals used for this experimental group, 4 animals died (28.6%, including 1 rat euthanized for poor health conditions). A Fisher's exact probability test confirmed that the difference in mortality between the lidocaine unshocked group and the remaining groups was statistically significant, $p < 0.05$. In order to achieve a balanced

design, with an equal number of subjects per cell, additional subjects were folded into the experimental groups whenever a subject died during the course of recovery.

Weight. Other indices of health also suggest that uncontrollable stimulation has an adverse effect on recovery. Prior to treatment, there were no significant differences in weight across groups within an experiment [F (1,36) < 1.0, p > 0.05]. As seen in Fig. 6, animals in the saline unshocked rats gained weight (+5.2 lbs.) over the 3-week recovery period, while subjects in the lidocaine shocked, lidocaine unshocked, saline shocked group lost weight (-4.5 lbs., -9.6 lbs., and -20.4 lbs., respectively). An ANCOVA conducted on weight change across days showed a significant effect of shock [F (11,385) =2.87, p < 0.005]., as well as a significant time X shock X lidocaine pretreatment interaction [F (11,385) = 5.43, p < 0.001]. No other differences reached significance [all F's < 1.57, p > 0.05].

Bladder Function. Fig. 7 illustrates that there were no differences in the recovery of bladder function that were associated with either shock treatment or pretreatment with lidocaine. Exposure to shock failed to significantly delay bladder recovery [F (1,36) < 1.0, p > 0.05]. There was not a significant main effect of lidocaine treatment, nor was there a significant shock X drug interaction [Both F's < 2.10, p > 0.05].

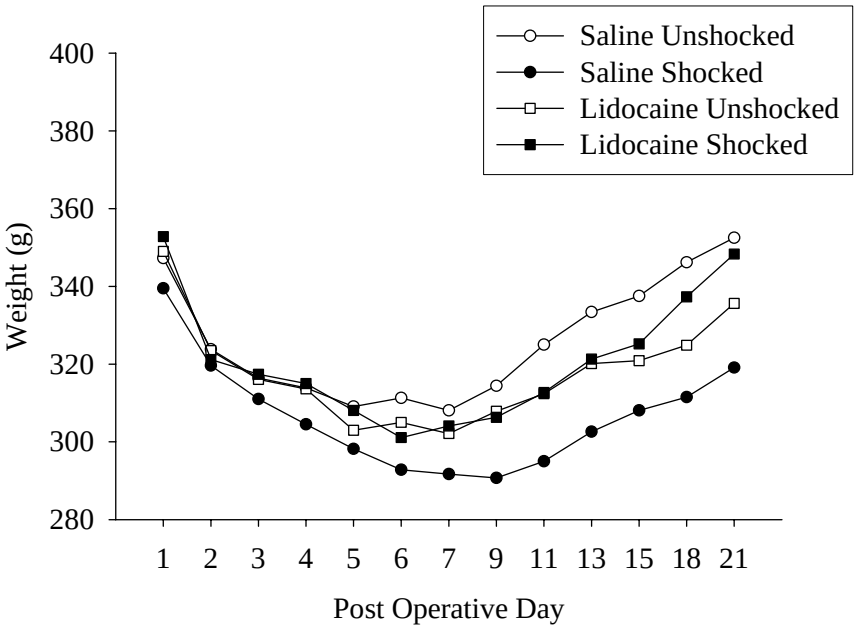

FIG. 6. Effect of lidocaine pretreatment and shock on weights throughout the recovery period

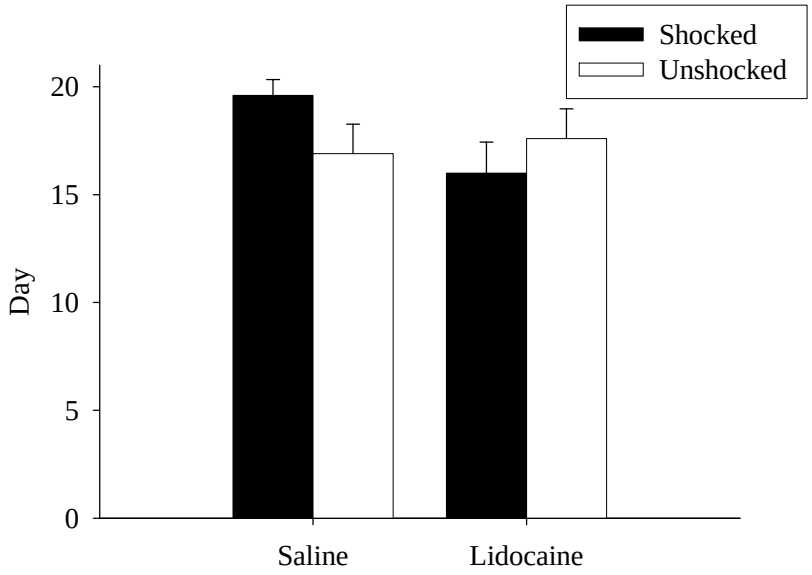

FIG. 7. Effect of shock and lidocaine pretreatment on recovery of bladder function

Spasticity. No animals in the saline unshocked group or the lidocaine shocked group exhibited spasticity (limb rigidity) during the three-week recovery period. In contrast, two saline shocked rats (20%) and two lidocaine unshocked rats (20%) exhibited spasticity. A Fisher's exact probability test failed to identify these differences as statictically significant, $p > 0.05$.

Autophagia. Some autophagic behavior was observed over the course of the recovery period. Only animals exposed to lidocaine pretreatment developed autophagic behavior. Three animals in the lidocaine shocked group (33.4%) and two animals in the lidocaine unshocked group (20%). No animals in either saline group developed autophagia. A similar amount of autophagia was observed in shocked (17.3%) and unshocked (15.0%) subjects. A contingency table testing the interaction between shock and drug treatment failed to reach significance, $p > 0.05$.

Sensory Function

Neither shock nor drug treatment had a significant impact on the threshold for eliciting a withdrawal response [both Fs < 1.43, $p > 0.05$]. There was also no interaction between shock and drug affecting withdrawal response [$F(1,31) = 2.24$, $p > 0.05$]. The one exception was the motor response engaged by a gradually incremented shock. Though there were no main effects of drug or shock [both Fs < 2.80, $p > 0.05$], there was a significant shock X drug interaction [$F(1,31) = 5.78$, $p < 0.05$]. As illustrated in Fig. 8, post hoc comparisons of the group means indicated that the lidocaine unshocked group took significantly longer to make a motor response to shock, $p < 0.05$. As can be seen in Fig. 8, saline shocked rats showed a trend in increasing latency to respond, no other differences approached significance, $p < 0.05$. An ANOVA revealed no significant main effects of shock or drug treatment, and no significant shock X drug interaction, on latency to vocalize to heat or shock [*all F's* < 1.21, $p > 0.05$].

Unlike previous studies, assessment of tactile reactivity revealed that neither shock nor drug treatment resulted in an increased latency to respond to a non-painful tactile stimulus [F (1,31) < 2.0, p > 0.05]. To assess sensitization or habituation had occurred across trials, an ANOVA was performed on the change scores across legs. There was no effect of test trials observed [F (1,31) = 3.62, p > 0.05].

Histological Analyses

Data were analyzed separately on the segment taken at the lesion center, and then on the collapsed average of three different sections (600 microns rostral to lesion center, lesion center, and 600 microns caudal to lesion center). As the formulas for calculating the relative percent values depend on utilizing a standardized width coefficient, an ANOVA was conducted on the raw widths to determine if there were differences in the size of spinal cords that varied systematically with drug treatment or shock condition. If the groups did not differ, ANOVAs were performed on the converted scores (relative % values). Otherwise, all analyses were performed on the raw (unconverted) scores.

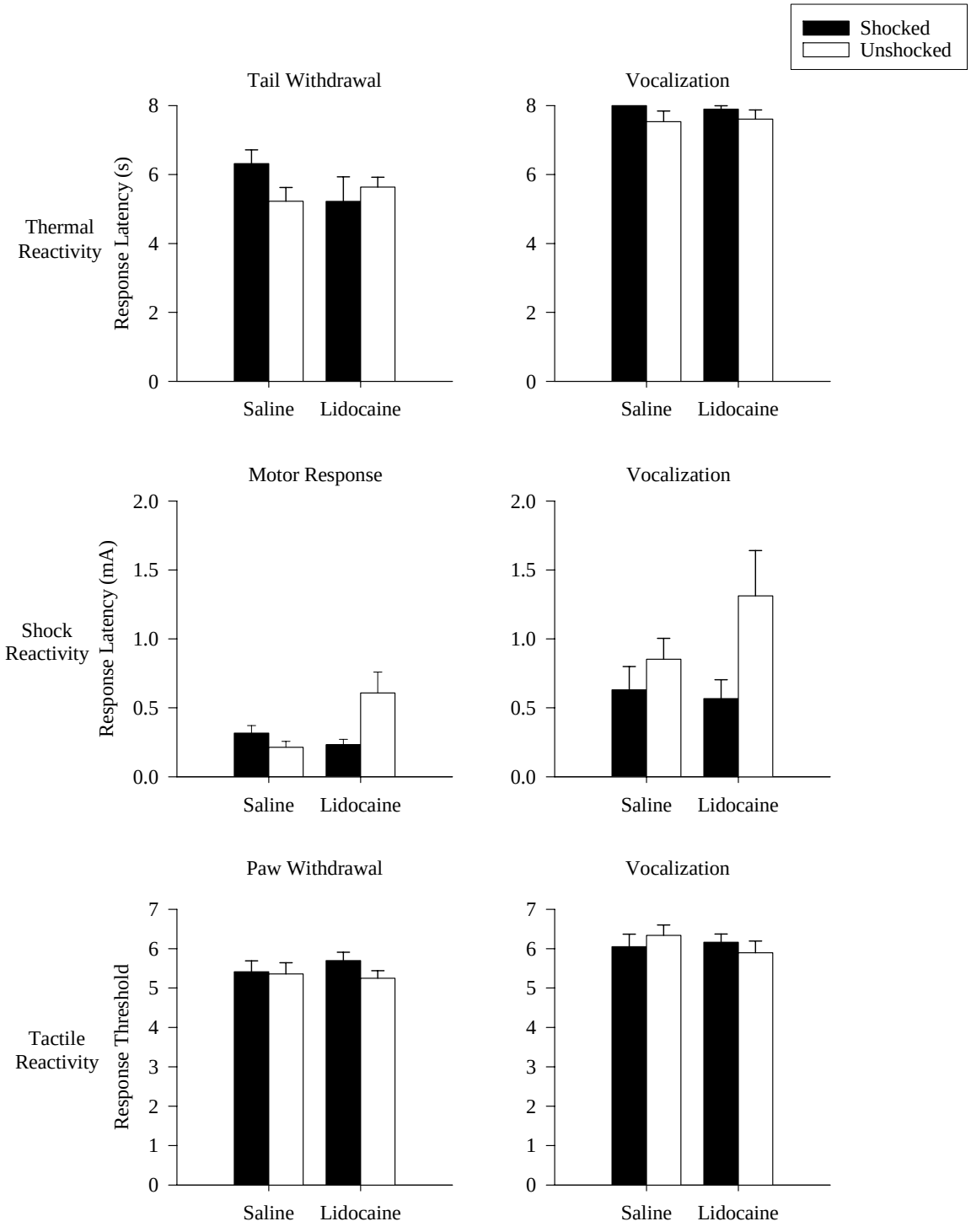

FIG. 8. Effect of shock and lidocaine pretreatment on latency to respond and vocalize to shock, heat, and tactile stimuli.

Lesion Center Tissue Analysis. Fig. 9 shows the effects of lidocaine pretreatment on the five histological outcome measures. An ANOVA on the raw width values for the lesion center confirmed a main effect of lidocaine pretreatment on width [F (1,35) = 5.69, p < 0.05]. As a result, all ANOVA's were run on the raw data measured in square millimeters for spared white, spared gray, total tissue, total area, and damage. There were no effects of lidocaine pretreatment or shock exposure on any of the histological outcome measures [all Fs < 3.03, p > 0.05].

Collapsed Tissue Analysis. An ANOVA on the raw width values for the collapsed (rostral, center, caudal) values identified no effect of lidocaine pretreatment or shock on width [F (1,35) = 3.27, p > 0.05]. Subsequent ANOVA's were then run on the corrected values expressed as a percent (% white, % gray, % lesion, % damage, and % missing). There were no effects of lidocaine pretreatment or shock on any of the histological outcome measures [all Fs < 1.25, p > 0.05].

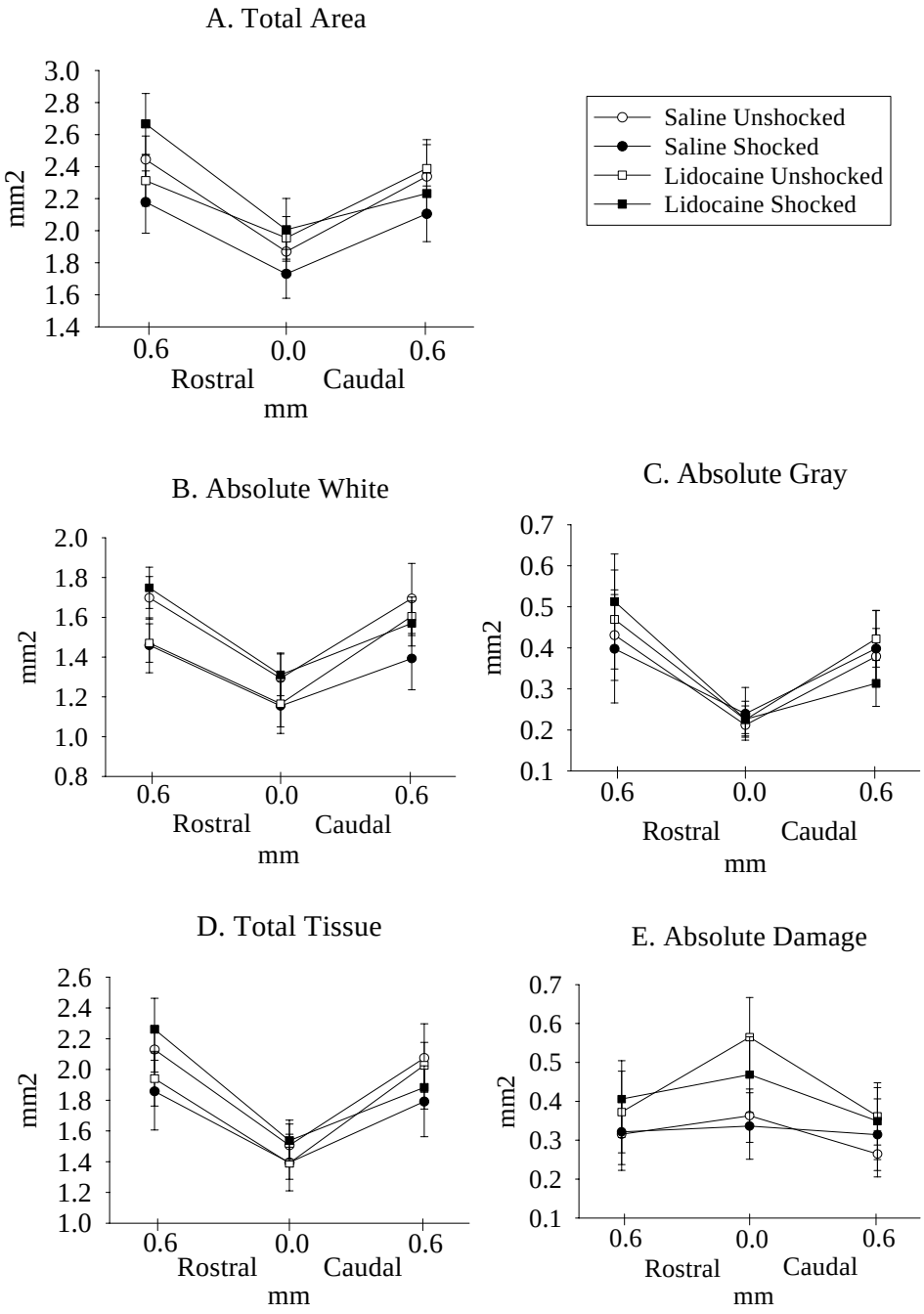

FIG. 9. The effect of shock and lidocaine pretreatment on tissue survival after a contusion injury

EXPERIMENT 3

Experiment 3 examined whether contused rats given uncontrollable stimulation while anesthetized would subsequently exhibit a deficit in recovery. We have previously shown that pentobarbital anesthesia does not affect the induction of the deficit in spinally transected rats (Washburn et al., 2002). Moreover, pretreatment with pentobarbital amplified the deficit in intact rats, presumably because it blocks the brain-dependent protection of spinal circuits (Washburn et al., 2004). Given these findings, if the deficit in recovery observed after uncontrollable stimulation reflects an alteration in spinal circuitry, contused rats given shock under pentobarbital should show a greater disruption of recovery. On the other hand, if the deficit reflects a brain dependent process, as the results from Experiment 2 suggest, pentobarbital anesthesia should have a protective effect.

Procedure

Twenty four rats were used in Experiment 3. The day following a contusion injury, subjects' locomotor performance was scored. Subject's Day 1 scores were counterbalanced to assign each subject to one of four conditions. A 2 (pentobarbital X saline) X 2 (shock X unshock) factorial design was used, consisting of four conditions: Pentobarbital Shocked, Pentobarbital Unshocked, Saline Shocked, Saline Unshocked. Approximately 24 hrs after contusion injury subjects were administered pentobarbital (50 mg/kg) given intraperitoneally (i.p.). Animals were then placed in their home cage for approximately 5 minutes. The individual giving the injections was blind to the experimental treatment. A tail pinch was used to assess both spinal reflexes and vocalization after animals appeared anesthetized. Subjects were considered functionally anesthetized if they had did not vocalize or retain spinal reflexes to tail pinch. Occasionally, subsequent supplemental doses of pentobarbital (0.05 ml) were used as boosters when animals failed to meet functional anesthesia criteria. Pentobarbital boosters

were given to 2 animals one time approx. 5-7 minutes after their initial dose, as which point functional anesthesia was reached. Animals were then placed in the shocking apparatus as described in the methods. Subjects then received either 30 minutes of uncontrollable tailshock or an equivalent period of restraint. Following shock treatment, rat's locomotor recovery was monitored using the BBB scale for 21 days. At the end of recovery all rats were assayed using the additional outcome measures as described in the methods.

Results

In accordance with results from Experiment 2, we found that pentobarbital anesthesia had a protective effect and blocked the adverse consequences of uncontrollable stimulation. The impact of pentobarbital treatment prior to shock exposure on locomotor recovery is illustrated in Fig. 10. The transformed BBB score values are given on the left y-axis.

Impact of Pentobarbital Pretreatment on Locomotor Recovery

It is clear that pentobarbital pretreatment protected animals from the adverse consequences of shock on recovery. An ANOVA confirmed that the behavioral scores on Day 1, prior to shock treatment, did not differ [F (1,19) < 1.0, p > 0.05]. A mixed ANCOVA (with Day 1 locomotor performance serving as the covariate) showed that pentobarbital pretreatment had a significant impact on overall performance [F (1,19) = 28.84, p < 0.001]. In addition, a significant shock X drug interaction indicated that pentobarbital treatment attenuated the effects of shock on recovery [F (1,19) = 10.94, p < 0.01]. Post hoc comparisons of the group means indicated that saline shocked animals had significantly lower average BBB scores compared to all other groups, p < 0.05. In addition, pentobarbital shocked animals had the highest BBB averages, and were significantly better than saline unshocked animals, p < 0.05. No other differences were significant, p > 0.05.

To verify that the groups differed at the end of behavioral testing, a separate ANCOVA was performed on the mean performance collapsed across the last 4 days of scoring (Days 13, 15, 18, 21). There was a significant main effect drug treatment [F (1,19) = 9.64, $p < 0.01$], as well as a significant shock X drug interaction [F (1,19) = 9.24, $p < 0.01$]. Post hoc comparisons indicated that the saline shocked animals had significantly lower BBB scores compared to all other groups, $p < 0.05$. No other differences approached significance, $p > 0.05$.

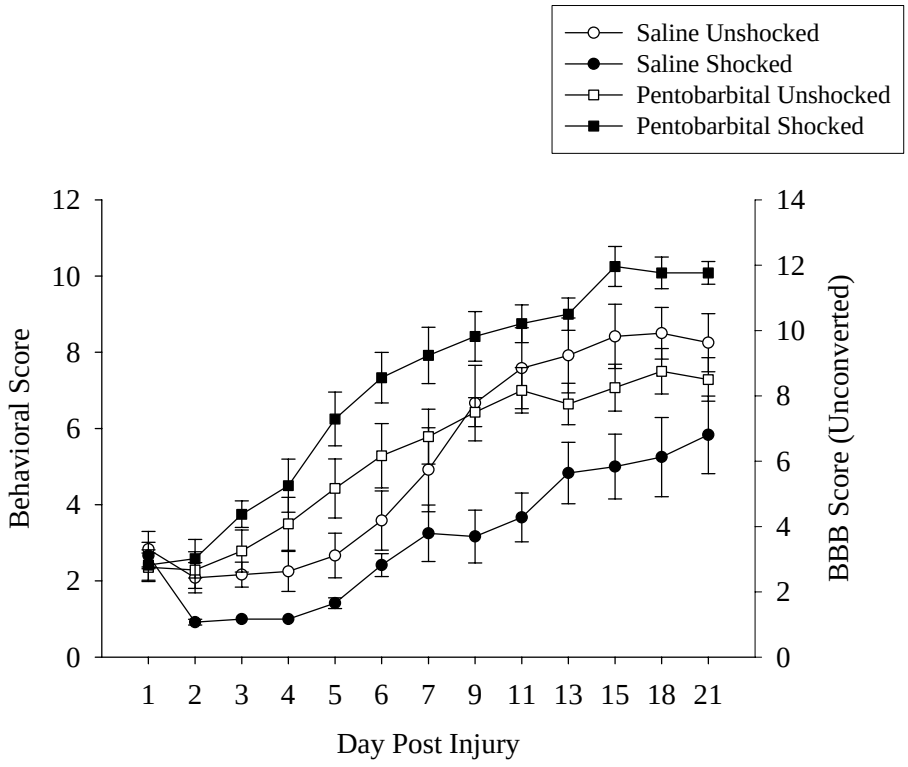

FIG. 10. Effect of pentobarbital pretreatment prior to shock on recovery of function

At the end of the recovery period, animals were tested on the ladder beam and the beamwalk tests. These tests provide convergent evidence of locomotor recovery and also

offer better assessments of postural control, balance, and coordination. An ANOVA conducted on the beamwalk data indicated that there was a significant effect of pentobarbital pretreatment [F (1,19) = 6.37, $p < 0.05$], as well as a marginally significant interaction [F (1,19) = 3.04, $p = 0.068$]. As seen in Fig. 11, shocked animals that received pentobarbital pretreatment were able to transverse narrower beams compared to saline treated animals. An ANOVA conducted on the ladderbeam data indicated that there were no main effects of pentobarbital pretreatment or shock [both $Fs < 3.36$, $p > 0.05$], but there was a significant interaction [F (1,19) = 5.07, $p < 0.05$]. Post hoc analyses indicated that saline shocked animals made significantly more errors on the ladderbeam when compared to the saline unshocked animals, $p < 0.05$ (Fig. 12). No other differences were significant, $p > 0.05$.

Other Indices of Recovery

Mortality. Subjects were assigned to the various experimental procedures 1 day after surgery. At this point, the probability of long-term survival was relatively high. No animals in the pentobarbital groups or the saline unshocked group died during recovery. However, one animal in the saline shocked group (16.4%) was euthanized due to poor health. A Fisher's exact probability test failed to find a significant difference in mortality between groups, $p > 0.05$.

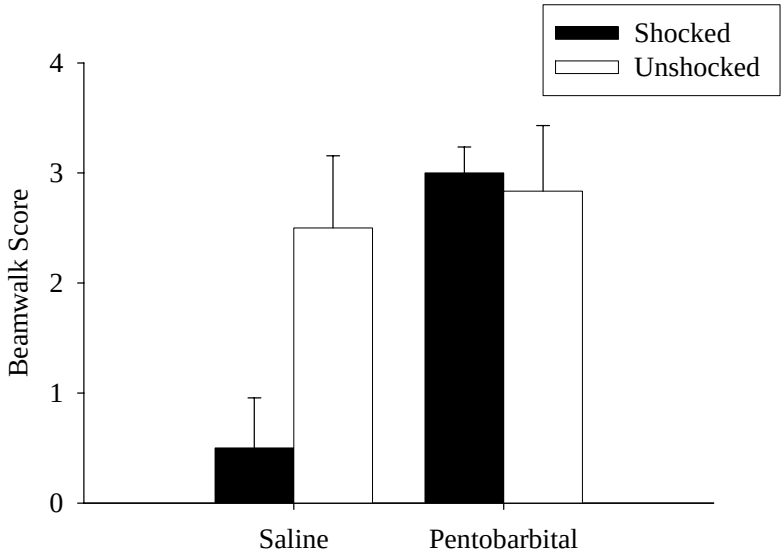

FIG. 11. Effect of pentobarbital pretreatment prior to shock on beamwalk performance

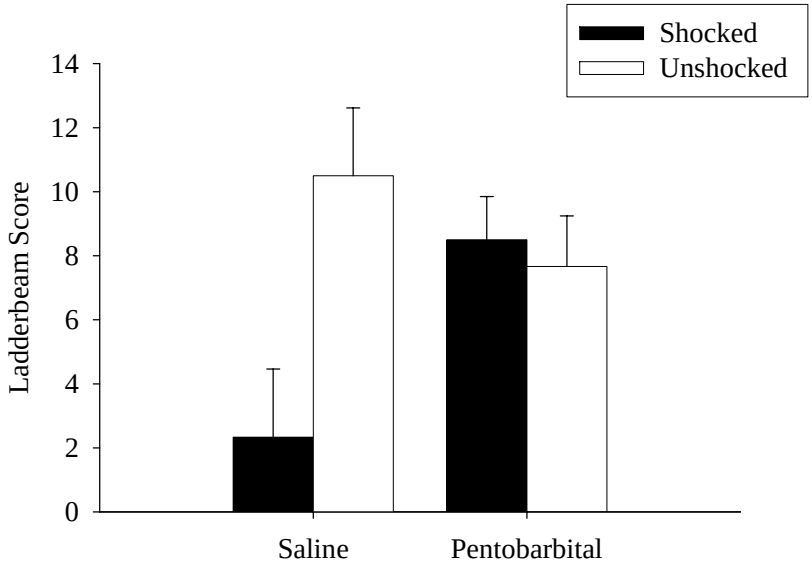

FIG. 12. Effect of pentobarbital pretreatment prior to shock on ladderbeam performance

Weight. Other indices of health also suggest that uncontrollable stimulation has an adverse effect on recovery. Prior to treatment, there were no significant differences in weight across groups within an experiment [F (1,19) < 1.0, p > 0.05]. Fig. 13 illustrates changes in weight across the recovery period. Pentobarbital treated subjects (shocked and unshocked) as well as saline unshocked rats gained weight over the 3-week recovery period, while subjects in the saline shocked group lost a significant amount of weight. An ANCOVA (using Day 1 weights as a covariate) conducted on weight change across days confirmed that there was a significant effect of pentobarbital pretreatment [F (1,18) = 7.424, p < 0.05]. Regardless of shock treatment, animals that received pentobarbital pretreatment gained the most weight over the recovery period compared to saline animals. No other differences approached significance, all p > 0.05.

Bladder Function. There were no differences in the recovery of bladder function that were associated with shock treatment. Fig. 14 shows the mean latency to recover bladder function. An ANOVA conducted to identify whether pentobarbital pretreatment attenuated the effects of shock on bladder function recovery failed to reach significance [F (1,19) = 1.77, p > 0.05]. No other differences were significant, p > 0.05.

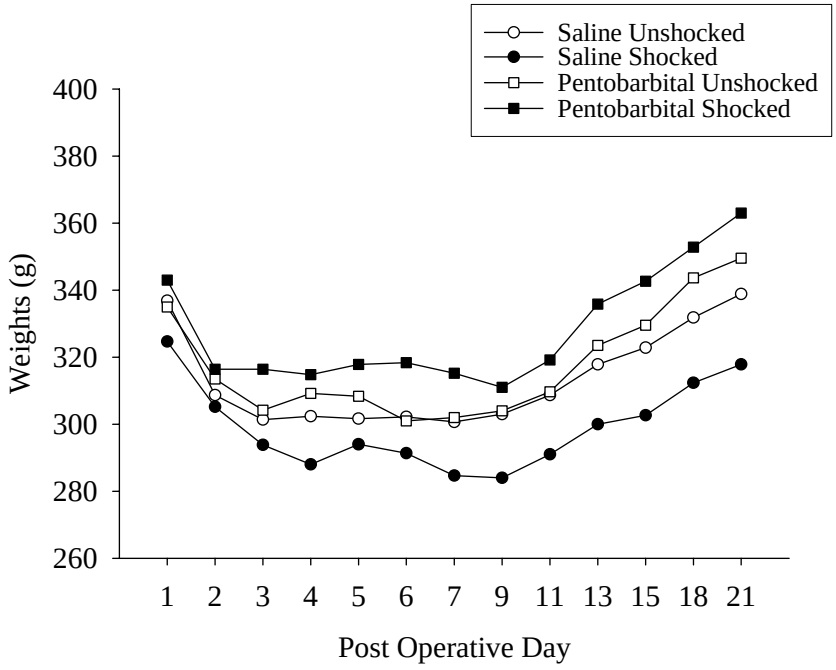

FIG. 13. Effect of pentobarbital pretreatment and shock on weights throughout the recovery period

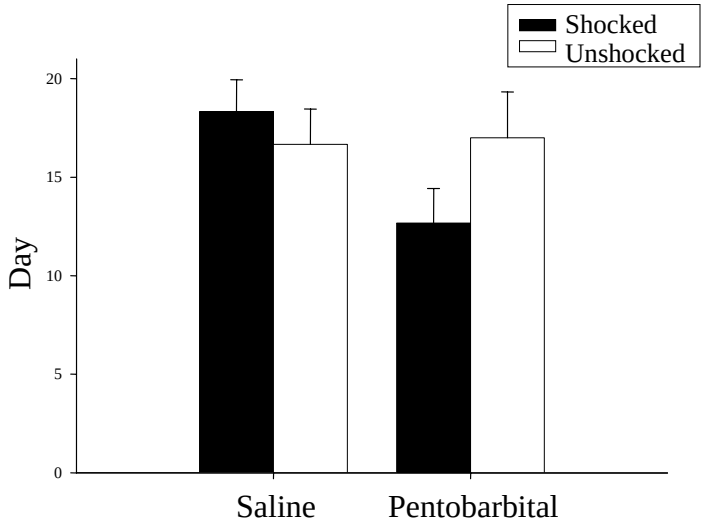

FIG. 14. Effect of pentobarbital pretreatment and shock on recovery of bladder function

Spasticity. No animals in the pentobarbital shocked group exhibited spasticity (limb rigidity) during the three-week recovery period. In contrast, one saline shocked rat (16.4%), one saline unshocked rat (16.4%), and one pentobarbital unshocked rat (16.4%) exhibited spasticity. A Fisher's exact probability test failed to identify these differences as statictically significant, $p > 0.05$.

Autophagia. Some autophagic behavior was observed over the course of the recovery period. One animal in the pentobarbital shocked group (16.4%) and one animal in the saline shocked group (16.4%) developed autophagia. No animals in either unshocked group exhibited signs of autophagia. A contingency table testing the interaction between shock and drug treatment failed to reach significance, $p > 0.05$.

Sensory Function

At the end of the recovery period, we assessed reactivity to an aversive shock, tactile stimulation, and a noxious thermal stimulus. In all cases, the stimuli were applied to a hindpaw or tail and over a range of intensities that normally elicit both a motor response (limb or tail withdrawal) and a supraspinally-mediated vocalization (King et al., 1996). Neither shock nor drug treatment affected the threshold for eliciting a withdrawal response [both Fs < 1.30, $p > .05$]. There was also no interaction between shock and drug affecting withdrawal response [$F(1,19) < 2.0$, $p > 0.05$]. There were also no effects of shock or drug treatment, and no interaction, on the motor response engaged by a gradually incremented shock [all Fs < 1.0, $p > 0.05$]. As can be seen in Fig. 15, saline shocked rats did show a trend in increasing latency to respond. An ANOVA conducted on latency to vocalize to heat and shock revealed no significant main effects of shock or drug treatment, and no significant shock X drug interaction, [all F's < 1.0, $p > 0.05$].

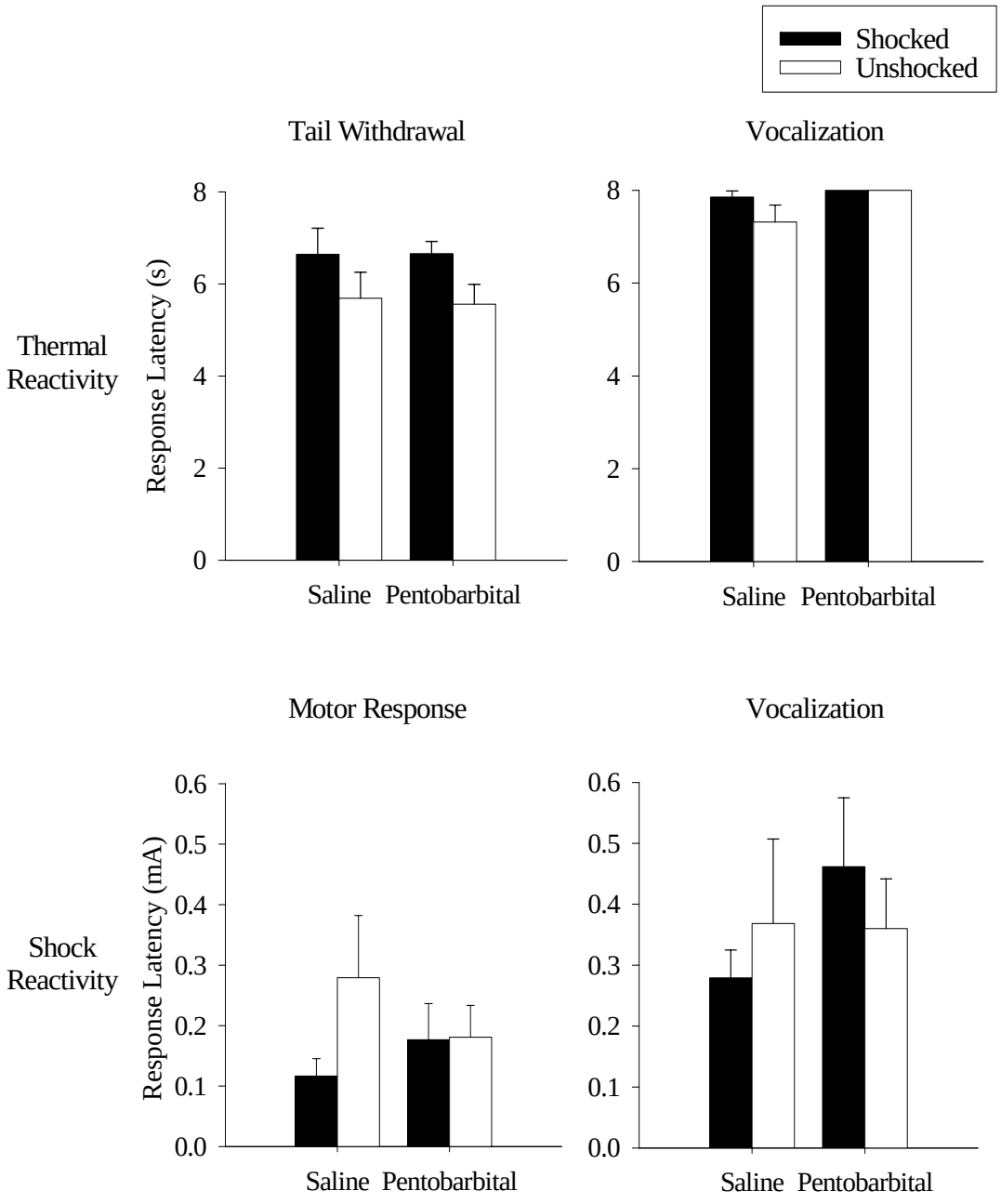

FIG. 15. Effect of shock and pentobarbital pretreatment on latency to respond and vocalize to shock and heat stimuli.

Histological Analyses

To identify whether pentobarbital pretreatment eliminated the loss in white and grey matter seen in previous experiments following shock treatment, ANOVAS were performed on the same histological outcome measures as described in Experiment 2. Fig. 16 shows that pentobarbital pretreatment attenuated the effects of shock on some of the outcome measures. An ANOVA confirmed there were no differences in width across conditions [F (1,20) < 1.0, $p > 0.05$]. ANOVA's conducted separately on the % white, % gray, % damage, % missing, and % lesion identified no effects of drug or shock treatment, and no interaction [all F's < 3.3, $p > 0.05$]. The effect of shock on the amount of missing tissue approached significance [F (1,20) = 3.3, $p = 0.08$].

An ANOVA conducted on the raw widths of the collapsed data (rostral, center, caudal) also identified no difference across groups [F (1,20) < 1.0, $p > 0.05$]. Additional ANOVA's to analyze the % white, % gray, % damage, % missing, and % lesion identified no effects of drug or shock on the amount of gray matter sparing, or the amount of lesioned or damaged tissue. A trend for unshocked animals to have increased white matter sparing (unshocked = 1.69 mm^2 vs. shocked = 1.52 mm^2) approached significance [F (1,20) = 3.5, $p = 0.076$], as did a trend for pentobarbital pretreated animals to have higher white matter sparing compared to saline treated animals (pentobarbital = 1.68 mm^2 vs. saline = 1.53 mm^2) [F (1,20) = 3.68, $p = 0.07$]. An ANOVA conducted on the percent of missing tissue showed that pentobarbital pretreated animals had significantly less missing tissue (21.7 % tissue missing) compared to saline treated animals (29.5% tissue missing) [F (1,20) = 5.12, $p < 0.05$]. (Missing tissue is calculated by subtracting the total tissue remaining (or total area) of the injured cord from the total area of a standardized slice corresponding to the same spinal segment in an uninjured cord) No other values approached significance, $p > 0.05$.

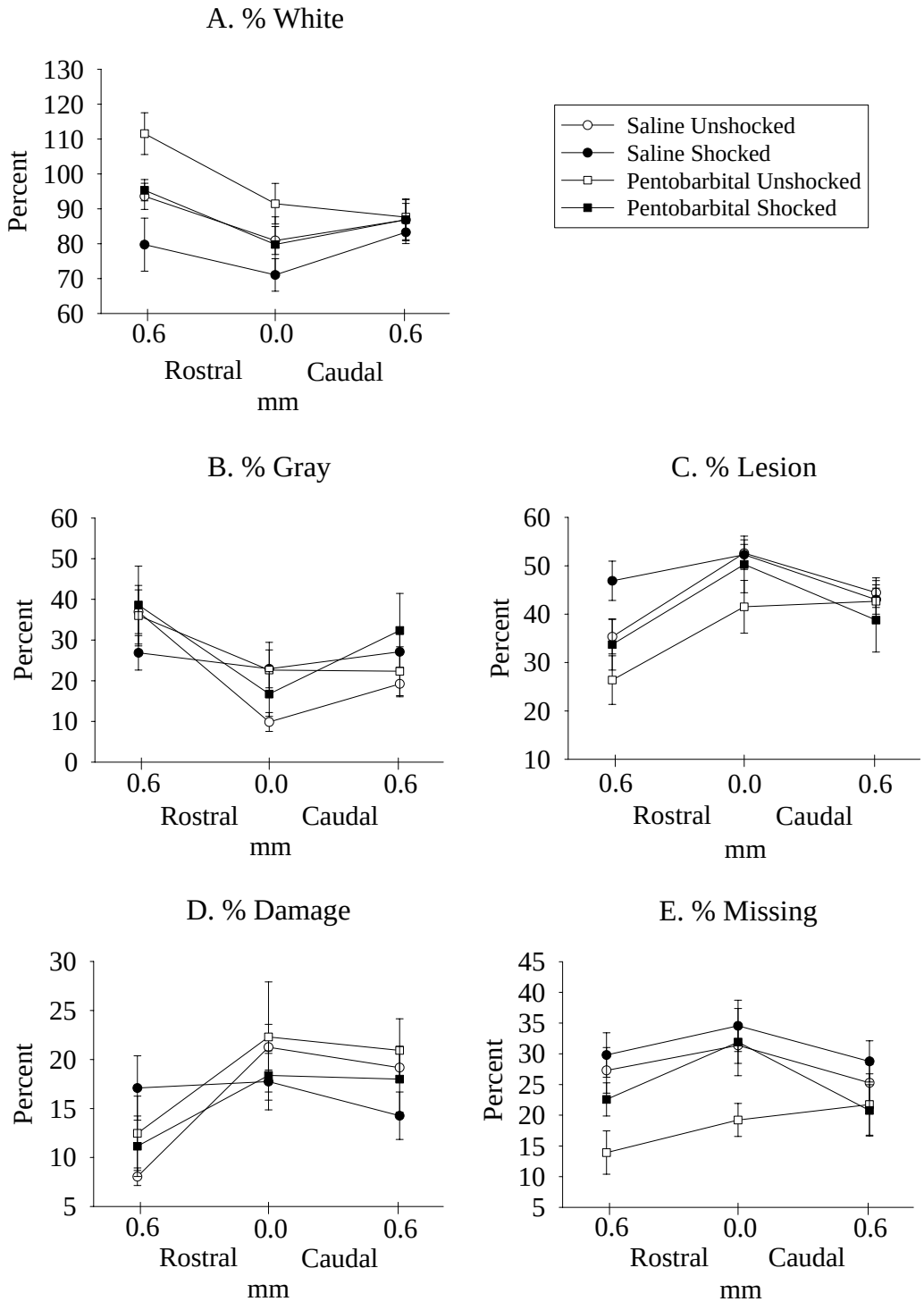

FIG. 16. The effect of shock and pentobarbital pretreatment on tissue survival after a contusion injury

GENERAL DISCUSSION

Uncontrollable nociceptive stimulation has been shown to have adverse consequences on both experimental models of learning in the spinal cord and clinically relevant models of spinal cord contusion injury. Up to this point, it has not been elucidated whether these adverse consequences reflect a brain- or spinally-mediated effect. Importantly, our preparation involves presentation of a shock stimulus caudal to the contusion injury that should minimize communication with the brain. However, even limited fiber sparing may provide an avenue for descending projections from the brain to attenuate the effects of the shock. The present experiments addressed these research questions by verifying that shock exposure induces an alteration within the spinal cord of contused rats and that disrupting communication between the spinal cord and brain during shock exposure protects animals from the effects of shock on recovery. Together, these findings shed light on some interesting interactions between the responses of different components of the CNS to injury.

We have previously shown that uncontrollable stimulation inhibits the capacity for instrumental learning in spinally transected rats. At this point, however, it is unclear whether uncontrollable stimulation would impact spinal neurons in contused animals. In line with our usual observations, Experiment 1 showed that contused rats transected prior to shock exposure failed to acquire the instrumental response when tested 24 hours later. Importantly, contused animals transected after shock exposure also failed to learn when tested, though this effect was less robust. We know that descending fibers that travel though the DLF protect intact animals from exhibiting the shock-induced spinal deficit (Crown & Grau, 2002). Given this, it is likely that the attenuated deficit observed in contused animals transected after shock resulted from fiber sparing after injury. These

results suggest that uncontrollable stimulation results in maladaptive changes in spinal cord plasticity in contused rats.

Experiment 1 showed that uncontrollable stimulation undermines spinal cord plasticity in contused rats. As such, it is plausible that this impaired spinal function is sufficient to explain the effects of shock on locomotor recovery. Experiments 2 and 3 addressed this possibility by manipulating communication between the brain and spinal cord prior to shock exposure. Experiment 2 utilized a concentrated dose of intrathecal lidocaine applied rostral to the injury to temporarily disrupt transmission. In Experiment 3, normal brain function was inhibited with i.p. pentobarbital. We replicated previous findings that vehicle treated animals exposed to shock demonstrate disrupted locomotor recovery. Interestingly, both manipulations showed that disrupting normal communication between the spinal cord and brain during shock exposure (by either disruption in brain function, or disruption of transmission from brain to spinal cord) protected animals from the adverse consequences of shock on locomotor recovery. Pretreatment with pentobarbital also protected animals from the harmful effects of shock on weight and recovery of bladder function. Investigation of histological outcomes indicated that animals pretreated with pentobarbital had significantly less missing tissue, shedding light on the potential role for brain mediated signaling in triggering necrosis and apoptosis in the spinal cord.

While Experiment 1 identified that shock results in changes in spinal cord plasticity, Experiments 2 and 3 point towards a more integrative hypothesis of brain mediated effects on spinal cord function. In intact subjects, descending modulatory systems function to diminish the negative impact of stressful events. When the spinal cord is injured these modulatory systems are compromised due to destructive cellular processes such as necrosis and apoptosis (Beattie et al., 2000, 2002; Crowe et al., 1997). The loss of

spinal cord neurons and axonal processes due to injury drastically alters the spinal cord environment (Shapiro, 1997). The data suggests that, in this compromised state, blocking communication between the brain and spinal cord protects animals from the adverse consequences of uncontrollable stimulation. In a state where the brainstem's descending inhibitory systems are compromised by injury, the brain's normally adaptive response to stress or trauma may actually be harmful.

The current experiments involving locomotor recovery following SCI replicated some of the key findings of our previous investigations of uncontrollable shock on recovery (e.g., Grau et al., 2004). As in Grau and colleagues (2004) uncontrollable shock disrupted locomotor recovery as assayed by the BBB scale. In addition, the current studies extended these findings by examining the impact of uncontrollable stimulation on other behavioral measures of postural control, balance, and coordination (beamwalk and ladderbeam). Experiments 2 and 3 indicated that animals exposed to uncontrollable shock made more errors (e.g., footfalls) on the ladderbeam test and were able to traverse only the widest beam during the beamwalk test. However, the current experiments failed to observe some of the effects of uncontrollable stimulation on recovery (e.g., bladder recovery, changes in weight, spasticity, histological outcomes) previously reported in Grau and colleagues (2004). Grau and colleagues (2004) used similar treatments across 3 experiments thereby gaining statistical power by collapsing the data. This design allowed those authors to observe some effects that were not statistically significant in the present report.

Learned Helplessness as a Model of Coping and Depression

Uncontrollable stimulation has been shown to produce learned helplessness in several models of learning (Crown & Grau, 2001; Grau et al., 1998; Maier & Seligman, 1976; Overmier & Seligman, 1967; Seligman & Maier, 1967; Weiss & Simson, 1986).

We have shown that the induction of helplessness with uncontrollable stimulation can have adverse effects on recovery after spinal cord injury. Previous work in our laboratory has shown that uncontrollable stimulation alters both behavioral measures of recovery and survival of spinal tissue (Grau et al., 2004). The present experiments suggest that, like learned helplessness in intact animals, the effects of uncontrollable stimulation after contusion injury are due to brain dependent processes (Grahn et al. 1999, Greenwood et al., 2005). These findings may indicate that psychological state could negatively affect functional recovery and tissue survival after spinal cord injury.

Interestingly, learned helplessness has been proposed as a model of human depression. More specifically, some suggest that the learned helplessness paradigm may actually be a good model for the effects of stress and coping on the manifestation of depression (Maier, 1984). It is not likely that stress results directly from exposure to a noxious or traumatic event, but instead, it has been proposed that distress is an outcome of the interplay between the painful event and psychological factors such as coping (Ursin, et al., 1978). An event becomes stressful when an organism cannot cope, or perhaps more interestingly, when an organism perceives that it will not be able to cope. In this way, the learned helplessness paradigm manipulates an organism's ability to make behavioral adjustments to control its environment, resulting in failed behavioral coping (Maier, 1984).

Incidence of Depression after Spinal Cord Injury

Depression is likely the most commonly researched psychological state in spinal cord injured (SCI) patients (Elliot & Umlauf, 1995). Studies report anywhere from 20 to 38 percent of acute SCI patients are depressed, with as many as half being diagnosed with Major Depressive Disorder (Judd et al., 1989; Kennedy & Rogers, 2000; Malec & Neimeyer, 1983). In addition, one study found that depression is not related to injury severity or extent (Malec & Neimeyer, 1983). Importantly, the incidence of depression in

SCI may be underrepresented as the studies reported are from acute injury time points. Reentry into the community including adjustment of family, career, and social life are likely sources of stress for SCI persons after the initial hospitalization and acute rehabilitation. Perhaps most relevant to our research is the finding that the lack of control over the event leading to injury is a significant factor in the development of long-term post traumatic stress disorder, depression, and subsequence life satisfaction after injury (Holbrook et al., 2005; Shultz & Decker, 1985).

Depression Affects Clinical Outcomes after Spinal Cord Injury

The current experiments suggest that psychological state could negatively impact the recovery of function after spinal cord contusion injury in rats. Investigation into the effects of psychological states such as depression have led to an overwhelming body of evidence supporting this hypothesis. It has been shown that high scores on measures of distress and depression can predict decreased bladder recovery and performance, as well as increased duration of inpatient rehabilitation (Malec & Neimeyer, 1983). Additionally, one study showed that patients exhibiting depressive behavior tend to have increased hospital stays and fewer functional improvements (Malec & Neimeyer, 1983). It has also been found that depressed SCI patients have an increased incidence of urinary tract infections and pressure ulcers (Herrick et al., 1994). This finding is especially important as chronic pressure ulcers contribute to increased incidence of infection, the third leading cause of death in SCI patients (National Spinal Cord Injury Statistical Center Annual Report, 2004). In line with the idea that even the perception of being unable to cope can result in stress, and thereby affect recovery, one study showed that patients who exhibit depressive symptomatology, when asked, expect longer hospitalization. Interestingly, these patients exhibit less functional independence and mobility at discharge (Umlauf & Frank, 1983). It should be noted that current body of research on depression after SCI is

limited in that many are only correlational, and often do not utilize statistical modeling techniques available to more concretely assess relationships between depression and clinical outcome. Nevertheless, these results provide support for the hypothesis that a psychological state such as depression could adversely impact recovery after SCI in the human population.

The present experiments raise questions regarding the relation of pre-existing psychological factors to recovery. If the effects we observed are simply another instance of learned helplessness, it might be expected that the induction of helplessness prior to a contusion injury would have similar adverse effects. We examined this possibility and found that exposure to uncontrollable stimulation adversely affects recovery only when the stimulus is presented after injury (unpublished observation). This finding highlights a key interaction between the time of exposure and its effect on recovery, and supports the idea that the brain's normal response to stress is only harmful when the spinal cord environment is altered due to injury. Further work is needed to identify whether this interaction depend on providing stimulation caudal to the injury.

Implications for Treatment after Spinal Cord Injury

Alleviation of chronic pain is the number one focus of persons with SCI (Hulsebosch, 2002). An average of 64% of people with SCI report pain sometime after injury, and as many as 40% report severe musculoskeletal pain beginning as early as two weeks following injury (Siddall et al., 1999). In addition, it has been shown that pain ratings and perception of pain severity are significantly correlated with depression (Cairns et al., 1996). The same study proposed a linear causality model of pain and depression, such that the experience of chronic and/or debilitating pain often leads to depression. Moreover, chronic pain has been shown to be associated with less functional recovery (Lundquist et al., 1991).

In light of all these findings, it seems logical that an appropriate treatment strategy after injury would be to target both pain and psychological state. It is natural to suppose that the adverse effects of uncontrollable stimulation are due to its aversive quality. This supposition predicts that opiate treatment would have a protective effect on recovery. We recently examined this issue and found that, as expected, pretreatment with i.p. morphine completely blocked behavioral reactivity to the shock stimulus (vocalization and motor reactivity). However, morphine did not protect animals from the adverse consequences of shock on recovery (Hook et al., submitted). Indeed, morphine treated rats exhibited a much higher level of mortality, suggesting that this treatment regimen should be avoided. Currently, we are testing other potential treatments and examining how brain and spinal processes interact to affect recovery.

In summary, the results of the current thesis have expanded our understanding of the interactions between the brain and spinal cord in the disruption of locomotor recovery that occurs following exposure to uncontrollable stimulation in SCI rats. These studies suggest that normal function of brain systems in protecting animals from uncontrollable stimulation is partially undermined by damage to the spinal cord caused by injury. When taken together with other work from our laboratory, these results highlight the need for novel treatments for pain and depression following central nervous system trauma.

REFERENCES

BASSO, D.M., BEATTIE, M.S., and BRESNAHAN, J.C. (1995). A sensitive and reliable locomotor rating scale for open field testing in rats. J. Neurotrauma **12,** 1-21.

BASSO, D.M., BEATTIE, M.S. and BRESNAHAN, J.C. (1996). Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. Exp. Neurol. **139**, 244-256.

BEATTIE, M.S., FAROOQUI, A.A., and BRESNAHAN, J.C. (2000). Review of current evidence for apoptosis after spinal cord injury. J. Neurotrauma **17**, 915-925.

BEATTIE, M.S., HERMANN, G.E., ROGERS, R.C., BRESNAHAN, J.C. (2002). Cell death in models of spinal cord injury. Prog. Brain Res. **137**, 37-47.

CAIRNS, D.M., ADKINS, R.H., and SCOTT, M.D. (1996). Pain and depression in acute traumatic spinal cord injury: Origins of chronic problematic pain. Arch. Phys. Med. Rehabil. **77**, 329-335.

CONSTANTINI, S., and YOUNG, W. (1994). The effects of methylprednisolone and ganglioside GM1 on acute spinal cord injury in rats. J. Neurosurg. **80,** 97-111.

CROW, M.J., BRESNAHAN, J.C., SHUMAN, S.L., MASTERS, J.N., and BEATTIE, M.S. (1997). Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys. Nat. Med. **3**, 73-76.

CROWN, E.D., FERGUSON, A.R., JOYNES, R.L., and GRAU, J.W. (2002a). Instrumental learning within the spinal cord: IV. Induction and retention of the behavioral deficit observed after noncontingent shock. Behav. Neurosci. **116**, 1032-1051.

CROWN, E.D., FERGUSON, A.R., JOYNES, R.L., and GRAU, J.W. (2002b). Instrumental learning within the spinal cord. II. Evidence for central mediation. Physiol. Behav. **77**, 259-267.

CROWN, E.D., and GRAU, J.W. (2001). Preserving and restoring behavioral potential within the spinal cord using an instrumental training paradigm. J. Neurophysiol. **86**, 845-855.

CROWN, E.D., and GRAU, J.W. (2002). Descending serotonergic systems protect spinal cord neurons from the deleterious effects of nociceptive stimulation. Program No. 363.3. 2002 Abstract Viewer/Itinerary Planner. Washington, DC: Soc. Neurosci. CD-ROM

CROWN, E. D. & GRAU, J. W. (submitted). Evidence that descending serotonergic systems protect behavioral plasticity against the disruptive effect of nociceptive stimulation. Exp. Neurol.

CROWN, E.D., KING, T.E., GRAU, J.W., and MEAGHER, M.W. (2000). Shock-induced hyperalgesia: III. Role of the bed nucleus of the stria terminalis and amygdaloid nuclei. Behav. Neurosci. **114**, 561-573.

ELLIOT, T.R., and UMLAUF, R. (1995). Measurement of personality and psychpathology following acquired disability. In: Cushman, L. & Scherer, M., editors. Psychological assessment in medical rehabilitation settings. Washington D.C.: American Psychological Association, 325-358.

FERGUSON, A.R., HOOK, M.A., GARCIA, G, BRESNAHAN, J.C., BEATTIE, M.S., and GRAU, J.W. (2004). A simple post hoc transformation that improves the metric properties of the BBB scale for rats with moderate to severe spinal cord injury. J. Neurotrauma **21**, 1601-1613.

GRAHN, R.E., WILL, M.J., HAMMACK, S.E., MASWOOD, S., MCQUEEN, M.B., WATKINS, L.R., & MAIER, S.F. (1999). Activation of serotonin-immunoreactive cells in the dorsal raphe nucleus in rats exposed to an uncontrollable stressor. Brain Res. **826**, 35-43.

GRAU, J.W., BARSTOW, D.G., and JOYNES, R.L. (1998). Instrumental learning within the spinal cord: I. Behavioral properties. Behav. Neurosci. **112,** 1366-1386.

GRAU, J.W., JOYNES, R.L., and PENLAND, H.R. (1996). Inescapable shock disrupts spinal learning: Evidence for spinal mediation and naltrexone reversibility. Soc. Neurosci Abs. **21**, 1840.

GRAU, J.W., WASHBURN, S.N., HOOK, M.A., et al. (2004). Uncontrollable stimulation undermines recovery after spinal cord injury. J. Neurotrauma **21**, 1795-1817.

GREENWOOD, B.N., FOLEY, T.E., BURHANS, D., MAIER, S.F., and FLESNER, M. (2005). The consequences of uncontrollable stress are sensitive to duration of prior wheel running. Brain Res. **1033**, 164-178.

HERRICK, S.M., ELLIOT, T.R., and CROW, F. (1994). Social support and the prediction of health complications among persons with spinal cord injuries. Rehabil. Psychol. **39**, 231-250.

HICKS, S.P., and D'AMATO, C.J. (1975). Motor-sensory cortex-corticospinal system and developing locomotion and placing in rats. Amer. J Anat. **143**, 1-42.

HOLBROOK, T.L., HOYT, D.B., COIMBRA, R., POTENZA, B., SISE, M., and ANDERSON, J.P. (2005). Long-term posttraumatic stress disorder persists after major trauma in adolescents: New data on risk factors and function outcome. J. Trauma **58**, 764-771.

HOOK, M.A., LIU, G.T., WASHBURN, S.N., FERGUSON, A.R., BOPP, A.C., et al. (submitted). Morphine undermines sensory recovery and increases histological damage after spinal cord injury. sent to J. Neurotrauma.

HORRIDGE, G.A. (1962). Learning of leg position by headless insects. Nature **193**, 697-698.

HULSEBOSCH, C.E. (2002). Recent advances in pathophysiology and treatment of spinal cord injury. Adv. Physiol. Educ. **26**, 238-255.

JOYNES, R.L., FERGUSON, A.R., CROWN, E.D., and GRAU, J.W. (2003). Instrumental learning within the spinal cord: V. Evidence that learning reflects an intraspinal modification. Behav. Brain Res. **141**, 159-170.

JOYNES, R.L., and GRAU, J.W. (2004). Instrumental learning within the spinal cord: III. Prior exposure to noncontingent shock induces a behavioral deficit that is blocked by an opioid antagonist. Neurobiol. Learn Mem. **82**, 35-51.

JUDD, F.K., STONE, J., WEBBER, J.E., BROWN, D.J., and BURROWS, G.D. (1989). Depression following spinal cord injury: A prospective in-patient study. Br. J. Psychia. **154**, 668-671.

KENNEDY, P., and ROGERS, B.A. (2000). Anxiety and depression after spinal cord injury: A longitudinal analysis. Arch. Phys. Med. Rehabil. **81**, 932-937.

KING, T.E., JOYNES, R.L., MEAGHER, M.W., and GRAU J.W. (1996). Impact of shock on pain reactivity: II. Evidence for enhanced pain. J. Exp. Psychol.: Anim. Behav. Proc. **22,** 265-278.

LIU, G.T., MIRANDA, R.C., WILKIE, J.M., WASHBURN, S.N., BOLDING, K.A, et al. (2003). Exposure to uncontrollable nociceptive stimulation increases vulnerability to apoptosis in spinally transected rats. 2003 Abstract Viewer/ Itinerary Planner. Washington, DC: Society for Neuroscience, 2003 CD-ROM.

LUNDQUIST, C., SIOSTEEN, A., BLOMSTRAND, C., LIND, B., and SULLIVAN, M. (1991). Spinal cord injuries: Clinical, functional, and emotional status. Spine **16**, 78-83.

MAIER, S.F. (1984). Learned helplessness and animal models of depression. Prog. Neuro-Psychopharmacol. Biol. Psychiat. **8**, 435-446.

MAIER, S.F., BUSCH, C.R., MASWOOD, S., GRAHN, R.E., and WATKINS, L.R. (1995a). The dorsal raphe nucleus is a site of action mediating the behavioral effects of the benzodiazepine receptor inverse agonist DMCM. Behav. Neurosci. **109**, 759-766.

MAIER, S.F., DAVIES, S., GRAU, J.W., et al. (1980). Opiate antagonists and long-term analgesic reaction induced by inescapable shock in rats. J. Comp. Physiol. Psychol. **94**, 1172-1183.

MAIER, S.F., GRAHN, R.E., and WATKINS, L.R. (1995b). 8-OH-DPAT microinjected in the region of the dorsal raphe nucleus blocks and reverses the enhancement of fear conditioning and interference with escape produced by exposure to inescapable shock. Behav. Neurosci. **109**, 404-412.

MAIER, S.F. and MINOR, T.R. (1993). Dissociation of interference with the speed and accuracy of escape produced by inescapable shock. Behav. Neurosci. **107**, 139-146.

MAIER, S.F., RYAN, S.M., BARKSDALE, C.M., and KALIN, N.H. (1986). Stressor controllability and the pituitary-adrenal system. Behav. Neurosci. **100**, 669-674.

MAIER, S.F. and SELIGMAN, M.E.P. (1976). Learned helplessness: Theory and evidence. Exp. Psychol. **105**, 3-46.

MALEC, J., and NEIMEYER, R. (1983). Psychologic prediction of duration of inpatient spinal cord injury rehabilitation and performance of self-care. Arc. Phys. Med. Rehab. **64**, 359-363.

MCLEMORE, S., CROWN, E.D., MEAGHER, M.W., and GRAU, J.W. (1999). Shock-induced hyperalgesia: II. Role of the dorsolateral periaqueductal gray. Behav. Neurosci. **112**, 539-549.

NATIONAL SPINAL CORD INJURY STATISTICAL CENTER (2004) Annual Statistical Report, June 2004, University of Alabama at Birmingham.

OVERMIER, J.B. (2002). On learned helplessness. Integr. Physiol. Behav. Sci. **37**, 4-8.

OVERMIER, J.B., and MURISON, R. (2000). Anxiety and helplessness in the face of stress predisposes, precipitates, and sustains gastric ulceration. Behav. Brain Res. **110**, 161-174.

OVERMIER, J.B., and SELIGMAN M.E.P. (1967). Effects of inescapable shock upon subsequent escape and avoidance learning. J. Comp. Physiol. Psychol. **63,** 28-33.

SANDI, C., BORRELL, J., and GUAZA, C. (1992). Behavioral factors in stress-induced immunomodulation. Behav. Brain Res. **48**, 95-98.

SELIGMAN, M.E.P. and MAIER, S.F. (1967). Failure to escape traumatic shock. J. Exper. Psychol. **74**, 1-9.

SELIGMAN, M.E.P., MAIER, S.F., and GEER, J.H. (1968). Alleviation of learned helplessness in the dog. J. Abnorm. Psychol. **73**, 256-262.

SELIGMAN, M.E.P., ROSELLINI, R.A. and KOZAK, M. (1975). Learned helplessness in the rat: Reversibility, time course, and immunization. J. Comp. Physiol. Psychol. **88**, 542-547.

SHAPIRO, S. (1997). Neurotransmission by neurons that use serotonin, noradrernaline, glutamate, glycine, and gamma-aminobutyric acid in normal and injured spinal cord. Neurosurg. **40**, 168-176.

SHULTZ, R. and DECKER, S. (1985). Long-term adjustment to physical disability: The role of social support, perceived control, and self-blame. J. Pers. Soc. Psychol. **48**, 1162-1172.

SIDDALL, P.J., TAYLOR, D.A., MCCLELLAND, J.M., RUTKOWSKI, S. and COUSINS, M.J. (1999). Pain report and the relationship of pain to physical factors in the first 6 months following spinal cord injury. Pain **81**, 187-197.

SOBLOSKY, J.S., COLGIN, L.L., CHORNEU-LANE D., DAVIDSON, J.F., and CAREY, M.E. (1997). Ladder beam and camera video recording system for evaluating forelimb and hindlimb deficits after sensorimotor cortex injury in rats. J. Neurosci. Methods. **78**, 75-83.

UMLAUF, R.L. and FRANK, R.G. (1983). A cluster-analytic description of patient subgroups in the rehabilitation setting. Rehabil. Psychol. **28**, 157-167.

URSIN, H., BAADE, E., and LEVINE, S. (1978). Psychobiology of stress. Academic Press: NY.

WASHBURN, S.N., FERGUSON, A.R., PATTON, B.C., and GRAU, J.W. (2002). Uncontrollable shock induces a behavioral deficit in the spinal cord: Role of GABA. 2002 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, 2002 CD-ROM.

WASHBURN, S.N., PATTON, B.C., FERGUSON, A.R., and GRAU, J.W. (2004). Exposure to uncontrollable stimulation during pentobarbital anesthesia has a long-term disruptive effect on spinal cord function. 2004 Abstract Viewer/Itinerary Planner. Washington, DC.: Society for Neuroscience, 2004 CD-ROM.

WATKINS, L.R., and MAIER, S.F. (2005). Immune regulation of central nervous system functions: From sickness responses to pathological pain. J. Intern. Med. **257**, 139-155.

WEISS, J.M., and SIMSON, P.G. (1986). Depression in an animal model: Focus on the locus ceruleus. Ciba Found. Symp. **123**, 191-215.

YAKSH, T.H., and RUDY, T.A. (1976). Chronic catheterization of the spinal cord subarachnoid space. Physiol. Behav. **17**, 1031-1036.

YOUNG, W. (2002). Spinal cord contusion models. Prog. Brain Res. **137**, 231– 255.

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Bopp. A. C., Ferguson, A. R., Liu, G. T., & Grau, J. W. (2004). Brain Dependent processes contribute to the adverse consequences of nociceptive stimulation on recovery of function. Washington DC: National Neurotrauma Society Annual Meeting, October, 2004.

Ferguson, A. R., Patton, B, C., Bopp, A. C., Meagher, M. & Grau, J. (2004). Brief exposure to a mild stressor enhances morphine-conditioned place preference in male rats. *Psychopharmacology*; 175(1): 47-52.

Hook, M. A., Liu, G., Washburn, S. N., Ferguson, A. R., Bopp, A. C., Huie, J. R., and Grau, J. W. (submitted). Morphine undermines sensory recovery and increases histological damage after spinal cord injury. sent to *Journal of Neurotrauma*.

Liu G. T., Ferguson A. R., Crown E. D., Bopp A. C., Miranda R. C., Grau J. W. (2005). Instrumental learning within the rat spinal cord: Localization of the essential neural circuit. *Behavioral Neuroscience*, 119(2): 538-47.